

Institute of Agronomy and Plant Breeding I
Justus Liebig University of Giessen

**Effect of different land uses (preceding crop/crop rotation x NPK fertilization)
on plant growth, biomass yields and on chemical and biological soil
parameters - results of a long-term experiment
“BNF” (Biological Nitrogen Fixation)**

Doctoral Thesis

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Content

Figures	I
Formulas	II
Tables	III
Abbreviations	VI
1 Introduction and objectives	1
1. 1 Introduction	1
1. 1. 1 Land use management and changes	1
1. 1. 2 Effects of land use management on soil properties and crop performance/quality	1
1. 1. 3 Characterization of long-term experiments (LTEs) as research tool to investigate the effects of land use management on soil	2
1. 1. 4 The LTE “Biological N Fixation” (BNF) Giessen	3
1. 2 Objectives of the study and hypotheses	4
2 Literature review	6
2. 1 Crop rotation	6
2. 2 Importance and functions of soil microorganisms	8
2. 3 Near surface soil CO ₂ emission (efflux)	10
2. 4 Importance of soil mesofauna	12
3 Material and Methods	15
3. 1 Site description	15
3. 1. 1 General information about the site	15
3. 1. 2 Climate conditions	15
3. 1. 3 Soil conditions	15
3. 2 Design of the LTE “Biological N Fixation”	17
3. 3 Plant parameters	18
3. 3. 1 Density of vegetative tillers and spikes	18
3. 3. 2 Plant height	19
3. 3. 3 Leaf area index (LAI)	19
3. 3. 4 Vegetation index (NDVI/NDRE)	19
3. 3. 5 Chlorophyll content of leaves (SPAD)	20
3. 3. 6 Biomass yield	20
3. 3. 7 Near infrared spectroscopy (NIRS)	21
3. 3. 8 Thousand grain weight (TGW)	21
3. 3. 9 Nitrogen use efficiency (NUE)	21

3. 3. 10 NPK analysis of plant samples	22
3. 3. 11 Potassium (K) and phosphorus (P) analysis	22
3. 4 Soil Parameters	24
3. 4. 1 Nitrate (NO ₃ ⁻) N analysis	24
3. 4. 2 Total carbon (Ct) & total nitrogen (Nt) analysis	26
3. 4. 3 Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) analysis	27
3. 4. 4 Total organic carbon (TOC) and total nitrogen bound (TNb)	27
3. 4. 5 Cation exchange capacity (CEC)	28
3. 4. 6 Microbial biomass carbon of the soil (SMB C)	29
3. 4. 7 Soil fauna feeding activity based on bait strips	30
3. 4. 8 Mesofauna abundance	30
3. 4. 9 Soil CO ₂ efflux measurement	32
3. 5 Statistical analyses	34
4 Results	35
4. 1 Effect of preceding crops and mineral NPK fertilization on soil parameters	35
4. 1. 1 Nitrate (NO ₃ ⁻) N content of the soil	35
4. 1. 2 Total Nitrogen (Nt) concentration of the soil	45
4. 1. 3 Dissolved organic nitrogen (DON)	50
4. 1. 4 Concentration of Total Nitrogen bound (TNb) in the soil	52
4. 1. 5 C/N ratio of the soil	54
4. 1. 6 Total Carbon (Ct) concentration of the soil	55
4. 1. 7 Dissolved organic carbon (DOC)	60
4. 1. 8 Total organic carbon (TOC)	61
4. 1. 9 Cation Exchange Capacity (CEC)	63
4. 1. 10 Soil microbial biomass carbon (SMB C)	63
4. 1. 11 Soil fauna feeding activity	66
4. 1. 12 Mesofauna (micro arthropods) of the soil	67
4. 1. 13 Soil surface CO ₂ efflux (Fc) dynamic	69
4. 2 Effect of preceding crops and mineral NPK fertilization on plant parameters	78
4. 2. 1 Number of vegetative tillers and spike density	78
4. 2. 2 Plant height	80
4. 2. 3 Leaf Area Index (LAI)	81
4. 2. 4 Vegetation Indices (NDVI/NDRE)	84

4. 2. 5 Chlorophyll content (SPAD) of the leaves	87
4. 2. 6 Biomass yield	90
4. 2. 7 Harvest Index (HI)	94
4. 2. 8 Thousand grain weight (TGW)	95
4. 2. 9 Nitrogen use efficiency (NUE _{crop} and NUE _{soil})	97
4. 2. 10 NPK uptake by the plants	99
4. 2. 11 Grain quality parameters (protein and starch)	103
5 Discussion	109
5. 1 Effect of preceding crops/rotational crop diversity and mineral NPK fertilization on soil parameters	109
5. 1. 1 Mineral nitrogen (NO ₃ ⁻)	109
5. 1. 2 Total nitrogen (Nt), total nitrogen bound (TNb) and dissolved organic nitrogen (DON)	111
5. 1. 3 Total carbon (Ct), total organic carbon (TOC), dissolved organic carbon (DOC), C/N ratio	114
5. 1. 4 CO ₂ efflux (Fc) of the soil surface	118
5. 1. 5 Cation exchange capacity (CEC)	122
5. 1. 6 Soil microbial biomass carbon (SMB C)	124
5. 1. 7 Mesofauna abundance and mesofauna feeding activity of the soil	127
5. 2 Effect of preceding crops/rotational crop diversity and mineral NPK fertilization on plant parameters of the subsequent crops winter wheat, winter rye and summer barley	130
5. 2. 1 Plant growth parameters (vegetative tillers, spikes, plant length)	130
5. 2. 2 Leaf chlorophyll (SPAD), Leaf area index (LAI) and vegetation indices (NDVI/NDRE)	132
5. 2. 3 Biomass yield, grain yield and harvest index	135
5. 2. 4 Nitrogen use efficiency and NPK uptake by winter wheat, winter rye and summer barley	140
5. 2. 5 Thousand grain weight (TGW) and grain quality (protein and starch content)	145
6 Validating of the hypothesis and conclusion	151
Summary	155
Zusammenfassung	157
Literature	160
Appendix	205
Acknowledgement	213

Figures

Figure 1: Location of the LTE “Biological nitrogen fixation”, experimental station “Weilburger Grenze” Giessen, GPS coordinates on Google Maps 2019	15
Figure 2: Development of air temperature (°C) and precipitation sum per month (mm) from 2015 to 2020, experimental station Giessen	16
Figure 3: Experimental design and treatments/plot distribution within the LTE “Biological Nitrogen Fixation” (since 1982) “BNF”– experimental station “Weilburger Grenze” Giessen	18
Figure 4: Soil sampling techniques (steel auger for drilling and taking soil sample)	24
Figure 5: Sampling device (Multiprob 120-UP) Nietfeld company, Experimental station “Weilburger Grenze” Giessen	24
Figure 6: Soil extraction and filtration method for nitrate (NO ₃ ⁻) N analysis, laboratory of the department of Agronomy	25
Figure 7: Drying chamber and sampling method to extract soil arthropods, high gradient technique, LTE-BNF	31
Figure 8: Soil CO ₂ efflux measurement by Licor-870 CO ₂ /H ₂ O infrared gas analyser-IRGA (SSA-1005) in LTE	32
Figure 9: Chamber-collar offset settlement for soil CO ₂ efflux measurement, LTE-BNF	32
Figure 10: The Stevens HydraProbe soil sensor	33
Figure 11: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO ₃ ⁻) N of the subsoil (60-90 cm) (September 2016), LTE “BNF” Giessen	37
Figure 12: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO ₃ ⁻) N of the subsoil (30-60 cm) (September 2016), LTE “BNF” Giessen	37
Figure 13: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO ₃ ⁻) N of the subsoil (30-60 cm) (September 2018), LTE “BNF” Giessen	37
Figure 14: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO ₃ ⁻) N of the subsoil (60-90 cm) (October 2019), LTE “BNF” Giessen	43
Figure 15: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO ₃ ⁻) N of the subsoil (60-90 cm) (April 2019), LTE “BNF” Giessen	44
Figure 16: Effect of pre-crops (PC) on DON of the topsoil (0-30 cm) March 2019, LTE “BNF” Giessen.....	52
Figure 17: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on TNb of the topsoil (0-30 cm) in March 2019, LTE “BNF” Giessen	53
Figure 18: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on DOC of the topsoil (0-30 cm) September 2018, LTE “BNF” Giessen	60

Figure 19: Mesofauna abundance (in %) in the soil (0-15 cm) in the LTE “BNF”, soil samples from August 2020, experimental station “Weilburger Grenze” Giessen	67
Figure 20: Micro arthropods (16 groups) identified in the soil (0-10 cm) in LTE “BNF” in August 2020, experimental station “Weilburger Grenze” Giessen	68
Figure 21: Dynamic of CO ₂ efflux (Fc) rate ($\mu\text{g C m}^{-2} \text{ s}^{-1}$) of the soil surface from Nov. 2018 till Jan. 2021, BNF-JLU Giessen	71
Figure 22: Seasonal variation of soil CO ₂ efflux (Fc) during cultivation of winter rye in 2020, LTE “BNF”, experimental station Weilburger Grenze, JLU Giessen	74
Figure 23: Monthly variation of soil surface CO ₂ efflux (Fc) during season 2020, LTE “BNF”, experimental station Weilburger Grenze, JLU Giessen	74
Figure 24: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on number of tillers in winter rye in 2016, LTE “BNF” Giessen	78
Figure 25: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on LAI of winter rye at DC 73 (grain fill period) in June 2016, LTE “BNF” Giessen	82
Figure 26: Effect of pre-crops (PC) on LAI in winter wheat 2019, LTE “BNF” Giessen	83
Figure 27: Effect of mineral fertilization (NPK) on LAI in winter wheat 2019, LTE “BNF” Giessen	83
Figure 28: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on chlorophyll content (SPAD) of leaves in winter wheat in 05.06.2015, LTE “BNF” Giessen	88
Figure 29: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on chlorophyll content (SPAD) of leaves in winter wheat in 16.06.2015, LTE “BNF” Giessen	88
Figure 30: Biomass yields of winter wheat depending on cropping system and mineral NPK fertilization, interaction effect between both factors, LTE “BNF” Giessen 2015	91
Figure 31: Effect of cropping systems on biomass yields (grain and straw) of winter wheat in 2019, LTE “BNF” Giessen	91
Figure 32: Effect of mineral NPK fertilization on biomass yields (grain and straw) of winter wheat in 2019, LTE “BNF” Giessen	92
Figure 33: Effect of cropping systems on biomass yields (grain and straw) of winter rye in 2016 (mean \pm SE), LTE “BNF” Giessen	92
Figure 34: Effect of mineral NPK fertilization on biomass yields (grain and straw) of winter rye in 2016, LTE “BNF” Giessen	93
Figure 35: Effect of mineral NPK fertilization on biomass yields (grain and straw) of winter rye in 2020, LTE “BNF” Giessen	93
Figure 36: Effect of pre crops on biomass yields (grain and straw) of summer barley in 2017, LTE “BNF” Giessen	94

Figure 37: Effect of mineral NPK fertilization on biomass yields (grain and straw) of summer barley in 2017, LTE “BNF” Giessen	94
Figure 38: Effect of pre-crops (PC) and mineral fertilization (NPK) on TGW of winter wheat in 2015, LTE “BNF” Giessen	96
Figure 39: Effect of pre-crops (PC) and mineral fertilization (NPK) on TGW of winter wheat in 2019, LTE “BNF” Giessen	97
Figure 40: Effect of pre-crops (PC) and mineral fertilization (NPK) on K uptake of winter rye in 2016, LTE “BNF” Giessen	103
Figure 41: Effect of pre-crops (PC) and mineral fertilization (NPK) on grain protein content (NIRS) of winter wheat in 2015, LTE “BNF” Giessen	104
Figure 42: Effect of pre-crops (PC) and mineral fertilization (NPK) on grain protein content (NIRS) of winter wheat in 2019, LTE “BNF” Giessen	105
Figure 43: Effect of pre-crops (PC) and mineral fertilization (NPK) on grain protein content (NIRS) of summer barley in 2017, LTE “BNF” Giessen	105

Appendix

Figure A. 1: Effect of pre-crops (PC) and mineral fertilization (NPK) on specific weight of winter wheat's grain in 2015, LTE "BNF" Giessen	211
Figure A. 2: Effect of pre-crops (PC) and mineral fertilization (NPK) on sedimentation value of winter wheat's grain in 2015, LTE "BNF" Giessen	211
Figure A. 3: Effect of pre-crops (PC) and mineral fertilization (NPK) on wet gluten of winter wheat's grain in 2015, LTE "BNF" Giessen	211
Figure A. 4: Effect of pre-crops (PC) and mineral fertilization (NPK) on sedimentation value of winter wheat's grain in 2019, LTE "BNF" Giessen	212
Figure A. 5: Effect of pre-crops (PC) and mineral fertilization (NPK) on specific weight of summer barley's grain in 2017, LTE "BNF" Giessen	212

Formulas

Equation 1: Normalized difference vegetation index	19
Equation 2: Calculation of dry matter determination at 60 °C	20
Equation 3: Calculation of dry matter determination at 105 °C	20
Equation 4: Calculation of dry matter - 100% total substance	20
Equation 5: Calculation of N use efficiency of crop	22
Equation 6: Calculation of N use efficiency of soil, based on N _{min} (NO ₃ ⁻) of the soil and the amount of N fertilized	22
Equation 7: Calculation of corrector factor for calibrating the Unicube (N _t measurement)	22
Equation 8: Calculation of nitrate (NO ₃ ⁻) N value (ppm) to mg Nitrate-N per 100 g soil	26
Equation 9: Calculation of nitrate (NO ₃ ⁻) N value (mg Nitrate-N per 100 g soil) to kg ha ⁻¹	26
Equation 10: Calculation of cation exchange capacity (CEC) in the soil	29
Equation 11: Calculation of soil dry matter in SMB C	29
Equation 12: Calculation of extinction value to dry matter of the soil in SMB C	29
Equation 13: Calculation of soil microbial biomass (SMB C)	30
Equation 14: CO ₂ efflux (F _c) calculation of the soil	32
Equation 15: Total volume calculation of the chamber, CO ₂ efflux measurement	33

Tables

Table 1: Mean value of air temperature (°C) and precipitation sum (mm) per month from 1982 to 2020, experimental station “Weilburger Grenze” Giessen	16
Table 2: Cultivation systems (CS) in the LTE “BNF” in Giessen since 1982, tenth rotational crop sequence in 2018-2021	17
Table 3: Concentrations of the K contained in the calibration solutions	23
Table 4: Concentrations of the P contained in the calibration solutions	23
Table 5: Concentrations of the nitrate (NO ₃ ⁻) N (µg/25 ml) contained in the calibration solutions	25
Table 6: Concentrations of the DON and DOC contained in the calibration solutions	27
Table 7: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO ₃ ⁻) N of the soil after harvesting of winter wheat in September 2015, LTE “BNF” Giessen	35
Table 8: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO ₃ ⁻) N of the soil after harvesting winter rye in September 2016, LTE “BNF” Giessen	36
Table 9: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO ₃ ⁻) N of the soil in September 2018, LTE “BNF” Giessen	38
Table 10: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO ₃ ⁻) N of the soil in November 2018, LTE “BNF” Giessen	40
Table 11: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO ₃ ⁻) N of the soil (March 2019), LTE “BNF” Giessen	41
Table 12: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO ₃ ⁻) N of the soil (October 2019), LTE “BNF” Giessen	42
Table 13: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO ₃ ⁻) N of the soil in April 2020, LTE “BNF” Giessen	44
Table 14: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt concentration of the soil (September 2015), LTE “BNF” Giessen	45
Table 15: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt level of the soil (0-30 cm) (September 2016), LTE “BNF” Giessen	46
Table 16: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt level of the soil (September 2018), LTE “BNF” Giessen	46
Table 17: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt concentration of the soil in November 2018, LTE “BNF” Giessen	47

Table 18: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt level of the soil in March 2019, LTE “BNF” Giessen	48
Table 19: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt concentration of the soil (October 2019), LTE “BNF” Giessen	49
Table 20: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt concentration of the soil in April 2020, LTE “BNF” Giessen	50
Table 21: Effect of preceding crops (PC) and NPK fertilization (NPK) on DON of the soil in September 2018, LTE “BNF” Giessen	51
Table 22: Effect of preceding crops (PC) and NPK fertilization (NPK) on DON of the topsoil (0-30 cm) March 2019, LTE “BNF” Giessen	52
Table 23: Effect of preceding crops (PC) and NPK fertilization (NPK) on TNb of the topsoil (0-30 cm) November 2018, LTE “BNF” Giessen	53
Table 24: Effect of preceding crops (PC) and NPK fertilization (NPK) on TNb concentration of the soil in March 2019, LTE “BNF” Giessen	54
Table 25: C/N ratio of the topsoil depending on pre crops and NPK fertilization over the time form Sept 2015 to April 2020, LTE “BNF” Giessen	55
Table 26: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct concentration of the soil in September 2015, LTE “BNF” Giessen	56
Table 27: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct concentration of the soil (0-30 cm) in September 2016, LTE “BNF” Giessen	56
Table 28: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct level of the soil in 2018, LTE “BNF” Giessen	57
Table 29: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct concentration of the soil in 2019, LTE “BNF” Giessen	58
Table 30: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct level of the soil in April 2020, LTE “BNF” Giessen	59
Table 31: Effect of preceding crops (PC) and NPK fertilization (NPK) on DOC of the soil (September 2018), LTE “BNF” Giessen	61
Table 32: Effect of preceding crops (PC) and NPK fertilization (NPK) on TOC of the topsoil (0-30 cm) November 2018, LTE “BNF” Giessen	61
Table 33: Effect of preceding crops (PC) and NPK fertilization (NPK) on TOC concentration of the soil in March 2019, LTE “BNF” Giessen	62
Table 34: Effect of preceding crops (PC) and NPK fertilization (NPK) on CEC of the topsoil (0-30 cm) in March 2019, LTE “BNF” Giessen	63
Table 35: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-30 cm) in September 2016, LTE “BNF” Giessen	64

Table 36: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-30 cm) in September 2018, LTE “BNF” Giessen	64
Table 37: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-30 cm) in October 2019, LTE “BNF” Giessen	65
Table 38: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-30 cm) in April 2020, LTE “BNF” Giessen	65
Table 39: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-15 and 15-30 cm) in October 2020, LTE “BNF” Giessen	66
Table 40: Effect of preceding crops (PC) and NPK fertilization (NPK) on soil fauna feeding activity in August 2020, LTE “BNF” Giessen	67
Table 41: Number of micro arthropods depending on pre-crops (PC) and NPK fertilization (NPK) in the soil, LTE “BNF” Weilburger Grenze Gießen 2020	69
Table 42: Effect of preceding crops (PC) and NPK fertilization (NPK) on CO ₂ efflux (Fc) of the soil surface in autumn 2018 and spring / summer 2019 during wheat growth cycle, LTE “BNF” Giessen	70
Table 43: Effects of preceding crops (PC) and NPK fertilization (NPK) on CO ₂ efflux (Fc) of the soil surface in the growing season 2020 (in winter rye from 04 Apr. to 17 Oct.) and in the winter period 2020 / 2021 (after plowing from 06 Nov. to 14 Jan.), LTE “BNF” Giessen	72
Table 44: Effect of preceding crops (PC) and NPK fertilization (NPK) on CO ₂ efflux (Fc) of the soil in the two vegetation periods in 2019 and 2020, LTE “BNF” Giessen	73
Table 45: C efflux of the soil surface per day, month and year in the LTE “BNF” Giessen, measurement campaigns 2018/2019 and 2020/2021	75
Table 46: Seasonal C/CO ₂ efflux of the soil surface in the LTE “BNF” experimental station Giessen 2018-2021	75
Table 47: Correlation matrix for the relationships between Fc and meteorological parameters, LTE-BNF 2019	76
Table 48: Correlation coefficients (r) based on linear regression for the relationship between CO ₂ efflux (Fc) and CO ₂ concentration of the air, air/soil temperature and soil moisture, BNF Giessen, 2018-2019	76
Table 49: Correlation matrix for the relationships between Fc and meteorological parameters, LTE BNF Giessen 2020	77
Table 50: Correlation coefficients (r) based on linear regression for the relationship between CO ₂ efflux (Fc) and CO ₂ concentration of the air, air/soil temperature and soil moisture, BNF Giessen, 2020-2021	77
Table 51: Effect of pre-crops (PC) and mineral fertilization (NPK) on number of tillers in winter rye 2016 and summer barley 2017, LTE “BNF” Giessen	79

Table 52: Effect of preceding crops (PC) and NPK fertilization (NPK) on spike density 2015-2020, LTE “BNF” Giessen	80
Table 53: Effect of preceding crops and NPK fertilization on plant height 2016-2020, LTE “BNF” Giessen	81
Table 54: Effect of preceding crops and NPK fertilization on LAI 2015-2016, LTE “BNF” Giessen	82
Table 55: Effect of preceding crops and NPK fertilization on LAI of winter rye 2020, LTE “BNF” Giessen	84
Table 56: Effect of preceding crops and NPK fertilization on NDVI of winter rye 2016, LTE “BNF” Giessen	85
Table 57: Effect of pre-crops and NPK fertilization on NDVI of summer barley 2017, LTE “BNF” Giessen	86
Table 58: Effect of pre-crops and NPK fertilization on NDVI of winter wheat 2019, LTE “BNF” Giessen	86
Table 59: Effect of preceding crops and NPK fertilization on NDVI of winter rye 2020, LTE “BNF” Giessen	87
Table 60: Effect of preceding crops and NPK fertilization on chlorophyll content (SPAD) of the wheat leaves in 2015, LTE “BNF” Giessen	87
Table 61: Effect of preceding crops and NPK fertilization on chlorophyll content (SPAD) of leaves in winter rye and summer barley (2016-2017), LTE “BNF” Giessen	89
Table 62: Effect of preceding crops and NPK fertilization on chlorophyll content (SPAD) of the leaves in winter wheat and winter rye (2019-2020), LTE “BNF” Giessen	90
Table 63: Harvest Indices (HI) of the subsequent crops winter wheat, winter rye and summer barley, LTE BNF Giessen 2015-2020	95
Table 64: Effect of preceding crops and NPK fertilization on TGW of winter wheat, winter rye and summer barley (2015-2020), LTE “BNF” Giessen	96
Table 65: N use efficiency of subsequent crops of winter wheat, winter rye and summer barley in kg/kg N within the 9 th (2015-2017) and 10 th (2019-2020) crop rotation	98
Table 66: N use efficiency of soil (NUE _{soil}) in kg yield/kg available N based on fertilizer and soil mineral N in winter wheat (2019) and winter rye (2020)	99
Table 67: Effect of preceding crops and NPK fertilization on N uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen	100
Table 68: Effect of preceding crops and NPK fertilization on P uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen	101
Table 69: Effect of preceding crops and NPK fertilization on K uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen	102

- Table 70:** Effect of preceding crops and NPK fertilization on grain protein content (NIRS) of winter wheat, winter rye and summer barley (2015-2020), LTE “BNF” Giessen104
- Table 71:** Effect of preceding crops and NPK fertilization on grain starch content (NIRS) of winter wheat, winter rye and summer barley (2015-2020), LTE “BNF” Giessen106
- Table 72:** Interaction effect between pre-crops (PC) and mineral fertilization (NPK) on grain starch content (NIRS) of winter wheat in 2015, LTE “BNF” Giessen107
- Table 73:** Interaction effect between pre-crops (PC) and mineral fertilization (NPK) on grain starch content (NIRS) of winter wheat in 2019, LTE “BNF” Giessen107
- Table 74:** Nmin (NO₃⁻) values (kg ha⁻¹) of the soil in the 9th rotation: 2015 (one year after preceding crops) and 2016 (two years after preceding crops) and in the 10th rotation: 2018 in the year of preceding crops cultivation, 2019 one year after pre crops109

Appendix

Table A. 1: Effect of preceding crops (PC) and NPK fertilization (NPK) on CO ₂ efflux (Fc) of the soil in vegetation periods in 2019, LTE “BNF” Giessen	205
Table A. 2: Effect of preceding crops and NPK fertilization on NDRE 2016, LTE “BNF” Giessen	205
Table A. 3: Effect of preceding crops and NPK fertilization on NDRE 2017, LTE “BNF” Giessen	206
Table A. 4: Effect of preceding crops and NPK fertilization on NDRE 2019, LTE “BNF” Giessen	206
Table A. 5: Effect of preceding crops and NPK fertilization on NDRE 2020, LTE “BNF” Giessen	207
Table A. 6: Effect of preceding crops and NPK fertilization on grain quality parameters in winter wheat 2015 and 2019, LTE “BNF” Giessen	207
Table A. 7: Effect of preceding crops and NPK fertilization on grain quality parameters in winter wheat 2015 and 2019, LTE “BNF” Giessen	208
Table A. 8: Biomass yield (grain and straw) of preceding crops-2018, LTE “BNF” Giessen	208
Table A. 9: Total N Biomass yield of preceding crops-2018, LTE “BNF” Giessen	209
Table A. 10: Effect of preceding crops and NPK fertilization on total Ca uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen	209
Table A. 11: Effect of preceding crops and NPK fertilization on total Mg uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen	210

Abbreviation

%	Percentage
°	Degree (angle)
°C	Degrees Celsius
µg	Microgram
µmol	Micromole
Al ³⁺	Aluminum
As	Arsenic metalloid
AT	Air temperature
Ba	Barium
BaCl ₂	Barium chloride
BNF	Biological Nitrogen Fixation
Ca	Calcium
CaCl ₂	Calcium chloride
CC	Crimson clover
Cd ³⁺	Cadmium
CEC	Cation exchange capacity
cm	Centimeter
cmolc	Centimoles of charge
CO ₂	Carbon dioxide
Cr ^{3+ / 6+}	Chrome
CT	Chamber temperature
Ct	Total carbon
Cu ²⁺	Copper
DC	Decimal code (development stage)
DM	Dry matter
DOC	Dissolved organic carbon (water soluble carbon)
DON	Water soluble nitrogen
dt	Decitone (volumetric unit)
DW	Dry weight
Ext	Extinction
FB	Field bean
F _c	Flux rate (efflux)
Fe ³⁺	Iron
g	Gram
Gi	Giessen
GLM	Generalized linear model
H ⁺	Hydrogen
ha ⁻¹	Hectare
HI	Harvest index
hl	Hectoliter
HNO ₃	Nitric acid

K	Potassium
$K_2S_2O_8$	Potassium peroxide sulfate
K_2SO_4	Potassium sulfate
kg	Kilogram
LAI	Leaf area index
LSD	Last significant differences
LTE	Long-term experiment
M	Maize
m^2	Square meter
Max.	Maximum
meq	Milliequivalents
Mg	Magnesium
mg	Milligram
$MgCl_2$	Magnesium chloride
Min.	Minimum
ml	Milliliter
mm	Millimeter
mol	Mole
N	Nitrogen
Na	Sodium
NDRE	Normalized difference red edge index
NDVI	Normalized difference vegetation index
$NH_4^+ N$	Ammonium
Ni^{2+}	Nickel
NIRS	Near infrared spectroscopy
nm	Nanometer
Nmin	Mineral nitrogen
NO	Nitrogen monoxide
$NO_3^- N$	Nitrate
NPK	Nitrogen, Phosphorous, Potassium
Nt	Total nitrogen
NUE	Nitrogen use efficiency
O	Oat
Obs.	Observation
P	Phosphors
PAR	Photosynthetically active radiation
Pb	Lead
PC	Preceding crop
ppm	part per million
s	Second (time unit)
S	Siemens (soil humidity unit)
SE	Standard error
SM	Soil moisture
SMB C	Soil microbial biomass carbon

SOC	Soil organic carbon
SPAD	Soil Plant Analysis Development
ST	Soil temperature
TGW	Thousand grain weight
TNb	Total nitrogen bound
TOC	Total organic carbon
w/w	Weight per weight (weight concentration of a solution)
yr	Year
Zn ²⁺	Zink

1 Introduction and objectives

1. 1 Introduction

1. 1. 1 Land use management and changes

The land use of the whole land area in Germany is primarily determined by agriculture, since around 50% of the entire area is used for agricultural production. Compared to that the areas for forestry (31%), for settlements (14%) and for waters (2%) are comparatively smaller (UBA, 2020). For this reason, the land use by agriculture is of great importance for the landscape and the environment.

About 70% of the total agricultural area in Germany is used as arable land on which cereal crops, root crops, rapeseed, fodder crops and legumes are grown in rotating crop sequences. Therefore, the methods used for crop management can directly or indirectly influence soil properties. These include soil tillage, organic and mineral fertilization as direct influencing factors or the choice of crop and crop rotation as well as sowing methods (mulch sowing), plant protection, irrigation and harvest methods as indirect influencing factors on soil properties.

Improper use and management of arable land that led to soil degradation are recognized as serious challenges for soil health and ecosystem sustainability (Lal, 2009). It has been declared that agricultural land use and management practices involving clearing of natural vegetation, over cultivation, and removal of crop residues have led to the greatest decline in soil organic carbon content (Negasa et al., 2017), and land susceptibility to water erosion risks (Fenta et al., 2019). In a poor management practice where the soil organic matter is limited, soil loss rates ranging from 37 to 246 t ha⁻¹ yr⁻¹ or total nitrogen loss of 48.81 kg ha⁻¹ yr⁻¹, depending on land use type, management practices and biophysical factors such as rainfall, vegetation cover and soil type (Adimassu et al., 2014). Hence, integrating crop diversity in a sustainable cropping system and management strategies can retrieve and maintain soil quality properties.

Rapid global changes due to changing land use, climate and non-native species are altering environmental conditions, resulting in more novel and sustainable cropping systems or land use managements. To understand how alternative scenarios of future land-use change and tackle agricultural markets and policies we need to involve more investigations to quantify and map potential drivers of proper cropping systems. Ecosystems with high biotic like habitat degradation, modification of soil properties, or climate change pose difficult questions for environmental management and research. Land use history has been a well-established field of research for several decades, and effects on vegetation and species distributions have been documented in different ecosystems following anthropogenic activities (Bruun et al., 2001).

1. 1. 2 Effects of land use management on soil properties and crop performance/quality

Land use and management practices are among the most important factors influencing key properties and ecological functions of soils. More recently, the effects of former land use on soil structure and chemistry have received increasing attention. Land use-induced gradients in nutrient availability may influence secondary succession, biomass production as well as

response to emissions of pollutants such as nitrogen (N), acidifying substances and greenhouse gases (Foster et al., 2003).

Conservation agriculture aims at reducing soil disturbance by ploughing, increasing input of organic amendments, diversifying the rotation and lengthening the period during which a crop is grown. These measures have a beneficial effect on the key indicators for soil health, be it biological properties (e. g. microbial biomass C, mean organic matter residence time, earthworms, soil enzymes, nematodes and pathogens, mycorrhizal fungi, soil respiration) or ecological functions (e. g. nutrient cycling, hydrological budget, energy budget, erosion, biodiversity, landscape processes; Lal, 2016).

Soil organic matter (SOM) is very dynamic and can change within decades to centuries (Richter, 2007). The potential of SOC storage depends on plant diversity and variability of biological properties (e. g. microorganisms, soil fauna, aggregation soil texture and type, natural vegetation, land use and management) and climatic properties (Wiesmeier et al., 2019). Changes in land use/cover types have impact on soil properties. SOC ensures soil quality by supplying nutrients, enhancing cation exchange capacity (CEC), supporting biodiversity, and improving soil aggregation and water-holding capacity (Bationo et al., 2007). The quantity of SOC varies spatially and temporally because of climatic, edaphic, biotic (flora, fauna and humans), topographical, and lithological factors, which influence the balance between the gains and losses of soil carbon. However, the greatest carbon fluxes between the atmosphere and the Earth's surface are attributed to anthropogenic factors, including land use and land cover changes (Lal, 2004; Smith, 2004 & 2008). Consequently, land cover change is a core theme of climate change research, which emphasizes the understanding of SOC responses to land cover dynamics.

Global change and elevated surface temperature can affect plants and their symbiotic microbial activity by influencing plant growth, health and ultimately crop yield (Jansen-Willems et al., 2016). Furthermore, the combination of elevated temperature and soil CO₂ respiration promote or mitigate microbiome and plant metabolism interactions (Campisano et al., 2017). The intensification of agricultural practices to increase food and feed outputs causing deterioration of soil quality and soil functions and land is becoming vulnerable to soil fertility declination and associated changes in physical and chemical properties. Hence, crop rotation, manuring, composting and no tillage followed by residue maintenance and biological nitrogen fixation can tackle some of these issues.

1. 1. 3 Characterization of long-term experiments (LTEs) as research tool to investigate the effects of land use management on soil

Long-term experiments (LTEs) are a valuable research infrastructure in agricultural research or climate change studies. They can provide valuable knowledge to study the long-term effects of different agronomic practices and climate variability to identify adaptation strategies in the proper cropping system management (Johnston and Poulton, 2018, Macholdt et al., 2020).

Increasing climate variability have been reported in Germany over past decades (Pompe et al., 2008; Wiebe et al., 2015). In contrary, there is broad agreement that conventional

management practices have adverse environmental impacts, with the potential of risking natural ecosystems over both the short and long terms (Duru et al., 2015). Water source scarcity, water contamination, air pollution and greenhouse gas emission, SOC depletion, soil erosion are the main drivers of this potential risk (Horrigan et al., 2002; Hurni et al., 2015). In general, management practices that increase agricultural production and reduce production risk also tend to support climate change adaptation because they increase agricultural resilience (Chazal and Rounsevell, 2009).

LTEs are the source of such information from which we can learn a lot about the factors that influence soils fertility and its sustainability. In most cases, effects and interactions can be understood only from LTEs data even if different soil and climate circumstances are compared to each other. Soil processes take place very slowly, therefore LTE trials are needed to investigate the influence of variable factors. LTE fertilization trials exploring both the yields of agricultural crops as well as the influence of fertilizers on the soil properties and soil indicators, the sustainability and the environment (Debreczeni and Körschens, 2003). Therefore, LTEs are an indispensable basis of our knowledge of predominantly practical solutions for sustainable land use.

LTEs represent a suitable agricultural research method to answer important soil, agronomic and ecological questions. With this method research questions about plant nutrient cycle and crop yield stability associated with climate change, can be answered, among other things (Merbach and Deubel, 2008; Reckling et al., 2018). Further on, the effect of agricultural management practices on soil characteristics like e.g soil organic carbon (SOC) (Ellerbrock and Gerke, 2016) as well as on physical, chemical and biological characteristics of the soil (Anglade et al., 2015; Sarker et al., 2018) can be examined. To this concept, the evaluation of the soil functions is a novel approach, which can be plausible only by LTEs (Vogel et al., 2019).

1. 1. 4 The LTE “Biological N Fixation” (BNF) Giessen

The current study was focussed on the LTE “Biological N Fixation” Giessen, which was established in 1982 and is still running. This LTE includes two factors.

First factor: The cultivation systems are expected to have different effects on the soil, which are likely to increase over time. The fallow land (no. 1), which is carried out every four years, will lead to a gradual degradation of the SOC and Nt in the soil, which cannot be compensated by the subsequent cultivation of the grain. In contrast, an increase in SOC and Nt as well as an improvement in soil biological parameters are expected in cultivation systems No. 2 and 3, which also have a positive effect on the biomass yields of the crops. In systems 4 and 5, long-term negative effects on soil fertility are expected, with system no. 5 (with maize) being assessed more negatively than system no. 4 (with oats).

Second factor: Each cultivation system was combined with four intensities of mineral fertilization: 1 without mineral fertilization, 2 with reduced dosage of PK fertilization, 3 with optimal level of PK and reduced N fertilization and 4 with PK and optimal N fertilization, whereby the effect of the cultivation systems on soil and crops could be strengthened or weakened.

1. 2 Objectives of the study and hypotheses

An open question is how sustainable these cultivation systems are on soil parameters and, also on biomass yields. This aspect can be examined by comparing different crop rotations or by analysing within the crop rotations which have already been running for a longer period of time, such as in the existing LTE "BNF". It has to be clarified whether the different land uses (fallow land, green mulch, field beans, oats, maize) can only be observed in the following year or whether these effects are also present in the third and fourth year of the rotation. If the latter is the case, we can assume a sustainable (lasting) effect.

In the ninth and tenth rotational crop sequence since the beginning of the LTE, clear effects of crop rotation and mineral NPK fertilization on relevant soil parameters and on plant biomass can be demonstrated. Based on the current state of knowledge and taking into account the conditions of the long-term experiment examined here, the following hypotheses are made regarding the effect of the test factors on soil properties and crop performance.

1. Cultivation systems with a proportion of 25% legumes within the crop rotation have led to an increase in the total organic carbon (TOC) and total carbon (Ct) content in the topsoil after at least nine crop rotations. This effect is highest in clover green mulch and lowest in fallow. It should be clarified whether this change has also occurred in the sub soil till 90 cm.
2. Cultivation systems with a proportion of 25% legumes within the crop rotation have led also to an increase of total nitrogen (Nt), total nitrogen bound (TNb) content, mineral nitrogen ($\text{NO}_3^- \text{N}$) and dissolved organic N compounds (DON) in the topsoil. This effect is highest in treatments with clover green mulch and lowest in soil of fallow land.
3. Legume crops including clover mulch and field bean increase the cation exchange capacity (CEC) of the topsoil compared to cereals or fallow land after at least nine crop rotations.
4. Year-round greening with clover mulch and annual field bean cultivation which were repeated at least nine times in the cultivation increase the soil microbial biomass carbon (SMB C) in the topsoil.
5. The CO_2 efflux of the soil is influenced by seasonal effects (soil temperature) and by soil moisture. The use of fallow land and legumes in a rotational crop sequence increase the CO_2 efflux of the soil surface in the first and second subsequent year.
6. Legumes (year-round green clover mulch and field beans) and mineral N fertilization led to an increase in the activity and abundance of the mesofauna of the topsoil layer in the ninth and tenth crop rotation, respectively.
7. The subsequent cereal crops react to the different previous crops and to the mineral N fertilization with an increase in vegetative growth indicated by higher leaf area index (LAI), vegetation indices (NDVI, NDRE), leaf chlorophyll (SPAD) and plant height.
8. Cultivation systems with a proportion of 25% legumes within the crop rotation have led to an increase of the biomass yields and harvest index (HI) in the first, second and third year of subsequent crops.

9. In addition to the direct effect of the mineral N fertilization, the legume previous crops also lead to a significant change in the grain quality indicated by an increase of thousand grain weight, protein content and volume weight but decrease of the grain starch content of the subsequent cereal crops.

2 Literature review

2. 1 Crop rotation

Agricultural cultivation systems are characterized by different crop rotations (rotational crop sequence) with varying plant diversity and intensities of mineral fertilization (especially with nitrogen). Depending on the site condition and duration, these factors can have an influence on soil health and on the biomass yields. Although these relationships have been researched frequently, they are still of great importance under current conditions of the global climate change and the need to develop resilient cultivation systems.

Crop rotation management may take advantage as essential tool in organic and conventional cropping systems as they contribute to ensuring its short-term productivity and long-term yield sustainability. Annual increasing of yield variability and declining potential yields of field crops due to climate change is an inevitable fact (Ray et al., 2015). Therefore, the relevance crop rotation studies lie in the fact that phenomena that take place at a slow rate like parameters of the soil fertility can be accurately examined, and recorded over a long time.

Crop rotation, mineral fertilization and further agronomic management practises like herbicide and pesticide application were implemented over decades to address the sustainable farming system by increasing yield performance (Johnston and Poulton, 2018). Many approaches in long-term studies confine to organic and inorganic fertilization, crop rotation systems and cover crops on soil functions such as carbon sequestration, carbon and nitrogen stock and micro-biological parameters (Herbst et al., 2016; Körschens et al., 2014). Diverse crop rotation systems are more effective in reducing long-term yield variability than monoculture systems (Macholdt et al., 2021), and can increase total carbon and nitrogen content of the soil over time, which may further improve soil productivity (Van Eerd et al., 2014).

Diverse crop rotation can sustain the beneficial soil organisms like soil microbes and soil mesofauna abundance by increasing the quality of crop residues, thus the coincidence of positive effects on SOM and soil fertility is inevitable (Marini et al., 2020; Tiemann et al., 2015). Many plant pathogens and non-mobile pests in the soil are particularly susceptible to the crop rotation (Peters et al., 2003). Therefore, different land use management (including legumes and cereals) with different plant phenotype and nutrition demands can break the chain of infection pathogens in the cropping system. As the cereals plant are the host of several saprophytic pathogens (Paulitz et al., 2010) introducing legumes to the cropping system can be a suitable biological control of plant diseases.

The sustainable farming system has often associated with cropping sequences, green manure, cover crops, mulches and crop residue management, which reported to reduce soil pathogens (Heather et al., 2006; Blok et al., 2000). Crop diversity in the management system especially those with higher phenolic compounds, provides appropriate soil microhabitats that allows complex microbial communities with different ecological functions to co-exist and enhance the soil fertility by developing disease suppressive soil (Weller et al., 2002).

Importance of cover crops in crop rotations

Benefits from “break crops” or pre-crops are more obvious in cereal rotations, where disease reduction can be a critical measure for yield benefitting factors of grain legumes. Thus, cereal-break crops management system is reported to yield on average 24% more than cereals grown continuously in Northern Europe (Kirkegaard et al., 2008).

Cereal yield increases following grain legumes as break crops associated with the increased N supply (Dalal et al., 1997). Similarly, higher yields of wheat after green manures compared with grain legumes (Silsbury, 1990) were also associated with increased soil nitrate concentrations, because the removal of N in harvested seed reduced crop effects on accretion of soil N. Using biological nitrogen fixation strategy reduces the need for inorganic or mineral nitrogen inputs and reduces the fossil energy resources required for mineral N production and indirectly decreasing CO₂ emission (Nemecek et al., 2008). Legumes also have the potential to increase the soil sequestered organic C and improve soil structure through deep rooting of species (Jensen et al., 2011). In addition, legumes are also able to solubilize soil phosphorous through releasing root exudates (Egle et al., 2003).

In addition, increased crop diversity, which is achieved by cultivation of legumes in rotational sequences, supports the richness and diversity of flora, fauna and soil microbiomes (Peoples et al., 2009). Further on, the effects of crop diversification can lead to pests and diseases being suppressed (Köpke and Nemecek, 2010).

Growing of cover crops is widely recommended for enhancing the soil fertility because of its major impact on soil microorganisms as well as soil arthropods communities. Thus, the increase of C and N accumulation in the soil by cover crops is associated with the biological N fixation, the amount of organic residues and root exudates, which in turn provides the proper soil condition for maintaining the soil fauna communities (Gamett et al., 2013; Giller et al., 2015; Williams et al., 2016). Furthermore, cover crops may improve soil aggregation, water holding capacity and reduce erosion. In addition, they can increase microbial biomass, improve weed suppression and prevent excess nutrient (Navarro-Noya et al., 2013; Teasdale, 2003).

Dual use of cover/green manure crops can serve as a main tool of soil nutrient management in organic farming strategies (Moore et al., 2016; Schonbeck et al., 2017). In another study, Scholberg et al. (2010) explained that deep-rooted and fast growing crops such as rye and crops with more root expansion can effectively acquire nutrients (especially N, but also P and micronutrients) from deep soil layers and make them more accessible for subsequent crops, when killed and incorporated or left as mulch.

The integration of cover and perennial legume crops and manure application contribute to increases of both soil organic C (SOC) and N (Kremer and Hezel, 2013). C availability in soil can increase over decades by the time, particularly in agricultural ecosystems, because of increased input of higher quality organic substances into the soil after harvesting (Geisseler and Scow, 2014).

Nitrogen losses (N₂O emission) decline in a diverse organic maize/soybean/winter wheat rotation compared to conventional-managed continuous maize system in Wisconsin (Osterholz

et al., 2014). Application of organic amendments (e.g., manure or compost) increase long-term C in crop management practices (Poulton et al., 2018). In addition, this can mitigate the residual nitrate N in the top 90 cm of soil in long term organic grain production compared to conventional systems, demonstrating a lower potential for leaching and under groundwater contamination (Baldock et al., 2014).

Water use efficiency is a big challenged in management of cropping systems, especially in the case of rain-fed cropping system. It has shown that the surface application of organic mulch layer like rye maintaining the moisture of the soil surface in the beginning of growing season when the crops are not well developed to cover the soil. They increase water infiltration into the soil, and provide pores and channels for roots to obtain water from the subsoil (Williams and Weil, 2004).

Plant root exudates contain components used in belowground chemical interactions (Massalha et al., 2017; Venturi and Fuqua, 2013). It is revealed that there are strong variations between different plant species and the soil microbiomes, which is suggesting that the exudates of the species are crucial in interaction of plants and microorganisms in the soil (Hartmann et al., 2009). Furthermore, the root residues are very essential in the establishment of the root microbiomes (Massalha et al., 2017).

In another study, it is explained that the root exudation changes are induced by nutrient limitation like phosphate (Ziegler et al., 2016). Therefore, implementing different crop species into the management system can reduce the nutrient limitation and recruit favourable soil microbiomes (Haney et al., 2015; Jacoby et al., 2017).

2. 2 Importance and functions of soil microorganisms

Soil microorganisms are the main drivers in C and N cycling, nutrient availability as well as in plant growth and plant productivity (Bardgett and van der Putten, 2014). In addition, soil microorganisms and root microbiomes have an important contribution to provide crop resilience in abiotic or nutrient stress confrontation as they govern the plant growing and development to their plant hosts by changes in root exudate (Hartman and Tringe, 2019). The capitalizing of the plant-beneficial soil microbes by plant's roots is a mechanism to reduce the abiotic stress. These plant-microbe associations occur at rhizosphere (Hartman et al., 2008).

The beneficial soil microbiomes also include plant growth promoting group of microbes, which primarily belongs to *Rhizobiaceae* that have symbiotic associations with leguminous plants by the formation of the root nodules. This symbiosis capability provides the ability of transforming of atmospheric N₂ into available organic N (reduction of one molecule N₂ to NH₃ with the help of the enzyme nitrogenase by prokaryotic microorganisms), which is known as biological N fixation (Oldroyd et al., 2011).

Furthermore, it is proved that the soil microbiomes not only contribute to biological N fixation but also mineralize organic and inorganic sources in the soil and transfer the nutrient into plant available form. For instance, P uptake and plant development in different species (Rodríguez and Fraga, 1999) or increasing N uptake and enhancing the drought tolerance by

arbuscular mycorrhizal fungi in their associated plant host have been reported (Vergara et al., 2017).

Inputs of organic C from crop residues combined with increased N availability provide a nutrient rich environment for microbial growth and may explain the higher relative bacterial abundances (Fierer et al., 2007). In convergence, few studies, which include bare land soils, have explained that the mineral nitrogen application to these soils has no effect on bacterial community composition compared with unfertilized soils (Yu et al., 2013).

Soil bacteria are efficient scavengers of inorganic N because they can exceed the mineralization by increasing the immobilization rates of the nutrient (Janssen, 1996; Bengtson and Bengtsson, 2005). It is also explained that the higher mineralization and immobilization coincidence with the higher microbial biomass and its activity (Zhang et al., 2000).

The microbial abundance is more strongly impacted by specific farming techniques, e. g. cover crop and green manure (Di Lonardo et al., 2019; Hopkins and Dungait, 2010). Changes in land use may altering microbial community structure in the soil (Rodrigues et al., 2013). Since soil, bacterial communities drive many different ecosystem functions, their abundance, richness, and composition they are therefore sensitive to the changes in the land use and management (Trivedi et al., 2016).

It is well known that the appropriate soil management practices on arable lands particularly under organic and mineral N resources, increase the soil organic carbon sequestration, which is beneficial for soil microorganisms and their activity, mainly due to a greater return of crop residues to the soil (Acosta-Martinez et al., 2007; Martyniuk et al., 2019).

It is also explained that the carbon measured as dissolved organic C (DOC) has a key role in multifunctionality of soil microbial composition and abundance (Bastida et al., 2016). In addition, the enrichment of the soil organic C sources like TOC proposed to decrease the soil suppressive pathogen by increasing the soil microbial communities, which is strongly linked to the land use and agricultural management (Bongiorno et al., 2019).

Earthworm populations are also favoured by surface crop residues and governs their decomposition rate. Therefore, they can profitably interact with soil microbial communities to reduce the soil-borne pathogens (Stephans et al., 1994), In addition, the quality of residues regarding carbon and nitrogen ratio is the qualitative factor to feed the soil microorganisms and prior to such earthworms. Thus, crop rotation including cover crops or preceding crops is important to regulate the soil health for feeding microorganisms (Brussaard et al. 2007; Malezieux, 2012).

In another study carried out on earthworms by van Groenigen et al. (2014) it is explained that the vegetation growth of crops under organic N fertilizer was (34%) higher than applying inorganic fertilizers (10%) or no fertilizer (20%) that can be attributed to the increase of N mineralization by earthworms and other microorganisms in the soil. Also, further studies indicating that earthworms have a high potential to increase N mineralization from residual organic matter in the cropping system depending to the environmental condition (Blair et al., 1997; Postma-Blaauw et al., 2006).

Furthermore, it has shown that crop residues like rye in soybean cultivation (Wen et al., 2017) may control soil suppressive diseases. Further on, incorporation of organic matter like green manure into the soil have been proposed to promote the soil aggregation and fertility (Cavigelli and Thien, 2003; Conklin et al., 2002) and decreases the occurrence of soil-borne pathogens (Noble and Coventry, 2005). Several studies have shown that adding the organic inputs to the cropping systems can effectively control the soil-borne diseases such as *Fusarium* sp. and *Rhizoctonia* sp. in wheat cultivation (Clapperton et al., 2001).

In addition, a reducing trend of microbial biomass in the soil of about 34% was observed in arable lands compared to the grasslands. The same tendency (21% reduction) was also found with regards to fungi communities in arable lands (Eekeren et al., 2008). Further on, the researchers observed also that the bacterial biomass was 50% higher in permanent grasslands compared to permanent arable lands. A similar tendency was found in soil quality monitoring in Germany, where bacterial biomass was about 50-100% higher in pasture compared to agricultural soil (Bloem et al., 2006). Also, in other studies it was shown that in croplands the SMB C level was reduced by 30% to 80% compared to fallow land (Wolińska et al., 2014; Wolińska 2019), which indicate the negative influence of bare arable soils on which no biomass input (root mass, aboveground biomass) and no soil cover occurs on soil microbial population.

2. 3 Near surface soil CO₂ emission (efflux)

Soil surface CO₂ efflux investigations can provide valuable information about C dynamics in the soil. Soil surface CO₂ efflux (F_s , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) is considered to be the largest component of the terrestrial ecosystem carbon, comprising 50 – 80% of ecosystem respiration (Davidson et al., 2002). Soil respiration is the sum of heterotrophic (R_h) and autotrophic (R_a) respiration in the soil, e.g. of roots, microbes, and soil fauna. It varies temporally, spatially on the plot and landscape level, and vertically with soil depth (Hogberg et al., 2001; Trumbore, 2000).

Increasing the atmospheric CO₂ concentration is the main factor that leads to global climate change (Scripps Institution of Oceanography, 2020). In this context, the agricultural ecosystems are prior because they can be either a net source or a net sink of CO₂ in the future. This depends on which CO₂ efflux overcomes the sequestered carbon from crop residues or root exudates down to the profile or releasing C by soil respiration. Significant increase of soil respiration by higher microbial activity and roots biological activity is proved (Biasi et al., 2008; Emmett et al., 2004; Wu et al., 2011a).

Additionally, fine root biomass, organic layer thickness (Arevalo et al., 2010; Paradiso et al., 2019), soil C and nutrient contents (Fan et al., 2015; Lai et al., 2012) or plant stand structure such as leaf or vegetation area index, canopy closure, leaf litterfall, leaf production, and mean annual precipitation can influence soil respiration (Reichstein et al., 2003; Tanaka and Hashimoto, 2006).

The land use strategy which is characterized by the input of crop residue and minimizing the C losses supposed to govern the CO₂ emission in the ago-ecosystem environment. Crop rotation by changing species result in the increase or decrease in CO₂ efflux from soils

(Woodbury et al., 2006; Trumbore, 2006). This, Han et al. (2013) explained that among different land use management systems (including cereals and legumes), soybean land has increased the accumulative CO₂ efflux by 26% during the growing season compared with maize land. In contrast, Bavin et al. (2009) showed that within a rotation system, the accumulative CO₂ efflux value from soybean land was about 40% on average lower than that from maize land applied with N fertilizers. These uncertainties associated with the differences in growing season of crop species or differences in application of mineral fertilization (Qiao et al., 2009).

Jacinthe et al. (2002) showed a positive directional effect of the soil mulching system on seasonal variation of CO₂ fluxes. Furthermore, it is showed that the soil CO₂ emission and mineralization of the organic C influenced by mulching system and is directly dependable to the soil temperature (Raich and Schlesinger, 1992; Franzluebbers et al., 1995; Fortin et al., 1996).

Soil respiration studies have recently been performed, focusing primarily on the differences in fertilization patterns based on long-term experiments (Li et al., 2009; Qiao et al., 2009). No fertilizer application in cropland led to the sharp decrease in soil CO₂ efflux, as indicated by previous studies conducted in the similar long-term fertilization experiments for different agro-ecosystems (Ding et al., 2007; Li et al., 2009). In convergence, Jacinthe et al. (2002) found inorganic nitrogen inputs have no significant effect on CO₂ fluxes. It is also explained that the decomposition of organic matter is not strongly dependable to the fertilization effect. As it is shown under such condition the soil stabilize the microbial activity through N mineralization. Similar results also reported in other studies (Corbeels et al., 2000; Qiao et al., 2009).

In addition, rain pulses in summer not only stimulate soil respiration (Borken et al., 2003; Lee et al., 2004; Liu et al., 2002) but can also lead to an increase in soil CO₂ concentration (Flechard et al., 2007; Jassal et al., 2005). Conversely, decreases of C emission by drought stress and reduction of above-ground biomass and nutrient availability to plant and microorganisms is reported (Pacific et al., 2009). Changing in SOC lead a directional impact on atmospheric CO₂ concentration (Lal, 2008a).

Several studies indicating the strong seasonal and environmental factors such as microclimate (air/soil temperature and soil humidity) as the major abiotic indices controlling CO₂ concentration. (Davidson et al., 2006; Liebig et al., 2013; Liikanen et al., 2006; Smith et al., 2003). They also concluded that the respiration rate was dependent on integrated influence of several factors like soil humidity and temperature, nutrient additions and microbial community (Fierer et al., 2003).

Soil moisture can alter soil CO₂ efflux by changing nutrient availability and by altering the composition and activity of decomposer microbes (Williams, 2007); for that reason, limiting soil moisture can suppress microbial activity independent of soil temperature and thereby the low correlations of soil CO₂ efflux to the temperature is plausible (Davidson and Janssens, 2006). Other studies demonstrated that soil moisture does not always statistically correlate with CO₂

efflux, although soil moisture is considered one of the important variables that help to drive soil C emission (Davidson et al., 1998; Suseela et al., 2012).

Climate conditions, land use and the soil management practices may affect the form and accumulation of SOC through the soil profile in agriculture systems (Bai et al., 2015; Wang et al., 2017). In arable land SOC, potentially confine to soil clay, the soil moisture and cultivation system can be improved by the mixed application of organic and inorganic fertilizers to the cropping system (Aguilera et al., 2013; Willaarts et al., 2016). Efficient utilization of crop residues and livestock manure in agricultural system not only decrease the expense of management system, but also improve the soil health by involving more organic material into the soil and protect the ecosystem by reducing CO₂ emission.

2. 4 Importance of soil mesofauna

The soil fauna can be characterized based on the size of the animals into macrofauna (> 2 mm, e. g. moles, gophers, earthworms, and millipedes), mesofauna (0.1–2 mm, e. g. springtails, mites and other arthropods) and microfauna (< 0.1 mm, e. g. nematodes and single-celled protozoans) (Weil and Brady, 2017). Although there are other definitions of the size of mesofauna animals however, the upper limit of 2 mm seems to be a generally valid value (Arribas et al., 2016). Together with the microfauna (size < 0.2 mm) and the macrofauna (size 2 - 20 mm), they form the entire soil fauna, which have important functions in the soil.

Arthropods as a major abundance of the soil represent 85 percent of the soil arthropods including Myriapoda, Collembola, Isopoda, Insecta and Acari (Culliney, 2013). The functions of mesofauna animals are to feed upon microorganisms and other soil animals (belonging to microfauna with size of about 2 to 100 µm), and to break down plant and animal material in the soil (Scheu et al., 2005). Further on, the mesofauna makes an important contribution to bioturbation, particularly through the litter fragmentation. In addition, by removing roots, they open drainage and aeration channels in the soil resulting in pore formation (Scheu et al., 2005).

The soil mesofauna can directly or indirectly enhance microbial activity, accelerate and regulate organic matter decomposition rates, increase soil respiration, and nutrient mineralization (Carrillo et al., 2011; Miller et al., 2017; Zhu Xinyu and Zhu Bo, 2015). For these reasons, arthropods belonging to the mesofauna can influence both the biological and physicochemical properties of the soil (Arribas et al., 2016).

In the study from Fu et al. (2009) was found, that the green mulching (clover) system or crop residues influences the abundance of soil fauna in farming system (Fu et al., 2009; Tremelling et al., 2003). It can be postulated that the components of crop residues among other crop species make the differences in quality of residues, for instance, legume mulch is more attractive to soil organisms (Birkhofer et al., 2011). Therefore, the quality of the organic matter effectively regulates and moderates the soil feeding activity by the soil fauna and microorganisms (Henriksen and Breland, 1999).

Soil fauna are a good indicator of the soil health, and it has been shown that the soil fauna can response directly to soil conditions (Vasconcellos et al., 2013). Since, the application of mineral inputs is necessary to promise enhanced crop yields (Diekötter et al., 2010), the

organic amendments and mineral N fertilizers influence soil fertility by rotating crop species, which directly influence the quality of residues provided by either of crop species, the root exudate and its expansion into the soil profile that significantly moderate the soil fauna populations and their regulations. For example, the higher abundance of soil micro-arthropods has been found after organic fertilization like straw and green manure treatments. (Reeve et al., 2010; Zhu Xinyu and Zhu Bo, 2015).

Furthermore, the management system indirectly influences the soil arthropod communities. For example, removal of vegetation can affect the soil pH and the available potassium in the soil. Thus, crop residue management suggesting the effects of the C/N ratio and SOC on the arthropod community (Birkhofer et al., 2008). For example, cereal straw with the higher C/N ratios caused low decomposition rates (Martens, 2000; Sieling, 2019) and reduced the attractiveness or palatability of straw for soil invertebrates (Ruiz et al., 2008).

In contrast, FYM and inorganic N fertilization showed no directional positive effect on population of arthropods (Brennan et al., 2006; Kautz et al., 2006; Zhu Xinyu and Zhu Bo, 2015). Furthermore, mineral fertilization directly affects the soil chemical properties, such as acidity, available nitrogen, and soil moisture, which have been proved to have a direct effect on micro-arthropods like collembolan (Silvertown et al., 2006) and soil microbial communities (Sun et al., 2015).

Evans and Sanderson (2017) reported that the long-term addition of mineral nitrogen fertilization could increase the arthropod community, which can attribute to the provision of a layer of organic matter developed on the soil surface. In addition, mineral fertilizations are essential to govern vegetation surface, therefore, it can also regulate microclimate like the soil moisture, and nutritional availability to plants (Schaffers et al., 2008). This result caused by increasing plant growth in NPK fertilizer treatments, which presumably caused an increase in microbial biomass, especially fungi, a major food resource of Collembola reported by Schütz et al. (2008). Some other practices showed heavy metals in agricultural soils have a negative impact on soil fauna (mites) abundance and richness (El-Sharabasy and Ibrahim, 2010; Migliorini et al., 2005).

Regarding this, the frequent utilization of inorganic fertilizers like phosphorous, raise the possibility of toxicity due to heavy metal accumulation in soil in the long-term fertilization system. The accumulation of the toxic elements in the soil increases the soil toxicity and can decrease the soil fertility (De Vries et al., 2013; Kabata-Pendias 2011). Heavy metals like cadmium (Cd), lead (Pb) and arsenic metalloid (As) have been found in P fertilizers and are considered the most important of health concern (Gupta et al., 2014). Moreover, it was pointed out that recycled P fertilizers were the main source of the heavy metals (Weissengruber et al., 2018).

Soil represents the most important reservoirs of biodiversity particularly in arable lands. The soil microbes and fauna diversity may vary over the years. For that reason, LTEs consider as a suitable tool for estimating the soil quality fluctuations and subsequently the sustainability of ecosystems. Within the complex structure of soil, improving in soil porosity and aeration as

well as the infiltration and decomposition of organic matter within the soil horizon addressed the potential benefits of soil organisms (Maharning et al., 2008; Menta, 2012).

In addition, the soil biological properties are depending to the microclimate conditions such as soil temperature and moisture and the LTEs supposed to be an appropriate measure of the soil biological functions among agricultural practices (Bongiorno et al., 2020). The long-term amendment of organic substances to the soil such as crop residues and green manure increase the organic carbon, which is linked to TOC and contribute as a main energy source for the soil microbiomes (Haynes, 2005). Indeed, conservation agriculture, which encompasses minimum soil disturbance, soil mulching and crop rotation, are in favor of soil fauna and their activity, which in turn improves the soil aggregation, carbon accumulation, proper nutrient cycle and water use efficiencies (Castellanos-Navarrete et al., 2012; Paul et al., 2015).

Furthermore, it was shown that the regulation of the soil arthropod communities is modified indirectly by the environmental condition such as soil/air temperature and precipitation. In fact, climate change governs the soil fauna by moderating their feeding activity through plant growth or the coverage of the soil (Kardol et al., 2010). In this regard Kardol et al. (2011) have shown a directional positive correlation among soil humidity and micro-arthropod richness, but not micro-arthropod abundance. Furthermore, collembolan abundance and richness positively responded to the soil moisture. While no significant relationships were distinguished for mites (Kardol et al., 2011).

3 Material and methods

3. 1 Site description

3. 1. 1 General information about the site

The long-term experiment “Biological Nitrogen Fixation” (BNF) is an ongoing trial, which was established in 1982 in the experimental station “Weilburger Grenze” Giessen (50° 36′ 12“ N, 8° 39′ 16“ E, 158 m a.s.l.) at the Justus Liebig University Giessen (figure 1). The total area of the experiment covers 7200 m² including the paths and edges.



Figure 1: Location of the LTE “Biological nitrogen fixation”, experimental station “Weilburger Grenze” Giessen, GPS coordinates on Google Maps 2019 (source: Google Maps, 2019).

3. 1. 2 Climate conditions

The climate (within the period 1982-2020) is characterized by a mean air temperature of 9.8 °C and a mean precipitation sum of 672 mm year⁻¹ (table 2). The experimental station Giessen is equipped with a proper weather station, which logs and records the meteorological data continuously over the entire time. The climate conditions of the site from 2015 to 2020 are presented in figure 2. It can be stated that the precipitation sum in the years 2016 to 2020 was higher than the long-term average in end of spring (May) to middle of summer (August). Furthermore, in 2015, 2018, 2019 and 2020 higher mean air temperatures (more than 20 °C, figure 2) were observed compared to the long-term average (table 1).

Table 1: Mean values of air temperature (°C) and mean precipitation sum (mm) per month from 1982 to 2020, experimental station “Weilburger Grenze” Giessen.

Month	Mean air temperature	Precipitation sum
	°C	mm
January	1.4	50.1
February	2.0	40.0
March	5.5	48.8
April	9.3	43.2
May	14.1	69.9
June	16.4	66.9
July	19.2	68.2
August	18.6	59.5
September	13.9	54.7
October	9.8	57.8
November	5.0	55.2
December	2.5	54.1

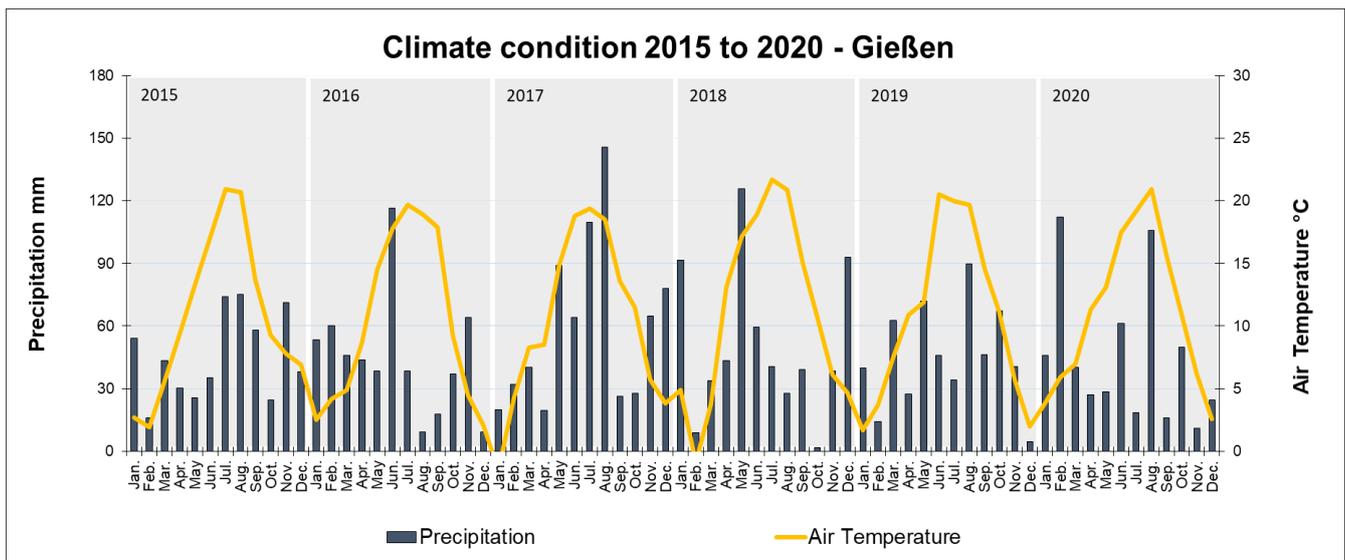


Figure 2: Development of air temperature (°C) and precipitation sum per month (mm) from 2015 to 2020, experimental station Giessen.

3. 1. 3 Soil conditions

The soil is classified as Eutric Fluvic Gleyic Cambisol (IUSS Working Group WRB, 2015) which is characterized (0-30 cm) by silty clay texture (39-49% clay, 40-58% silt, 4-12% sand). The usable field capacity (0-100 cm) of the soil is about of 123 mm. In the top soil (0-30 cm) the SOC varies from 0.7 to 1.6%; with a soil density of 1.2-1.3 g cm⁻³ (Hobley et al., 2018) and a pH value of 6.0-6.4. The K, P and Mg concentrations in the soil (from 2015 to 2018, based on VDLUFA method) can be characterized as following: potassium (K_{CAL}) level of 4-15 (mg/100 g soil), phosphorous (P_{CAL}) level of 4-18 (mg/100 g soil) and magnesium level of 9-28 (mg/100 g soil).

3. 2 Design of the LTE “Biological N Fixation”

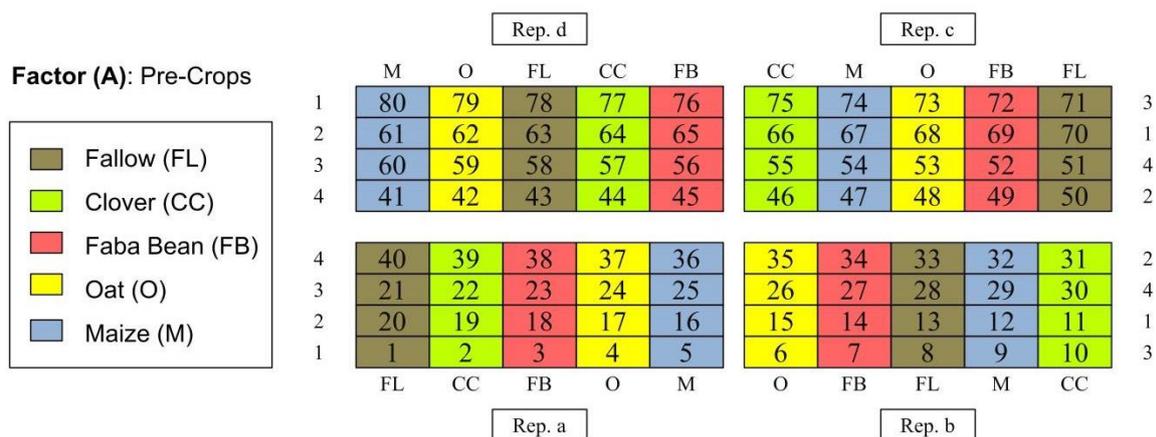
The field experiment includes two main factors (A) pre-crop/land use and (B) mineral fertilization (N, P, K) arranged as a randomized block design with four field replications. The four years rotational crop sequence is as follows: first year: five different pre-crops/land uses arranged parallel to each other in the same field including crimson clover (CC) (*Trifolium incarnatum* cv. Kardinal) used as green mulch, field bean (FB) (*Vicia faba* cv. Hiverna), summer oat (O) (*Avena sativa* cv. Fleuron), maize (M) (*Zea maize* cv. Sunshinos) and fallow land (F). Different pre-crops took places in the rotation for the last two times in 2014 and 2018 (table 2).

Crimson clover as green mulch and straw residues of fava bean, oat and maize were incorporated into the soil three weeks after harvesting. Second year: cultivation of winter wheat (cv. Premio and Rubisko, respectively) as the first subsequent crop after all pre-crops (in 2015 and 2019), Third year: cultivation of winter rye (cv. Brasetto and KWS Binotto, respectively) as the second subsequent crop (in 2016 and 2020). Fourth year: cultivation of summer barley (cv. Avalon) as the third and last subsequent crop within the rotational crop sequence (in 2017).

Table 2: Cultivation systems (CS) in the LTE “BNF” in Giessen since 1982, tenth rotational crop sequence in 2018-2021.

Cropping system	CS 1	CS 2	CS 3	CS 4	CS 5
Cereals	75%	75%	75%	100%	75%
Legumes	0%	25%	25%	0%	0%
Fallow	25%	0%	0%	0%	0%
Maize	0%	0%	0%	0%	25%
Rotational crop sequence					
1 st year	Fallow	Green mulch	Fava bean	Oat	Maize
2 nd year	Wheat	Wheat	Wheat	Wheat	Wheat
3 rd year	Rye	Rye	Rye	Rye	Rye
4 th year	Barley	Barley	Barley	Barley	Barley

Mineral fertilization to winter wheat, winter rye and summer barley includes four sub-treatments: (1) zero NPK, (2) only PK 50%, (3) PK 100%+N 50% dosage (90, 60 and 60 kg N/ha for wheat, rye and barley, respectively) and (4) PK+N 100% dosage (180, 120 and 90 kg N/ha for wheat, rye and barley, respectively). The plots cover a gross size at sowing of 80 m² and at harvesting a net plot size of 42 m². The LTE includes 20 treatments (5 pre-crops x 4 NPK fertilisations) and 80 plots (figure 3).



Factor (B): Mineral Fertilization

1. Without NPK
2. P 45 kg/ha K 60 kg/ha
3. P 90 kg/ha K 120 kg/ha +N %50 40+30+20 kg/ha
4. P 90 kg/ha K 120 kg/ha +N %100 80+60+40 kg/ha

- First year: Pre-crops / land uses
 Second year: Winter wheat
 Third year: Winter rye
 Fourth year: Summer barley

Figure 3: Experimental design and treatments/plot distribution within the LTE “Biological Nitrogen Fixation” (since 1982) “BNF”– experimental station “Weilburger Grenze” Giessen.

Nitrogen fertilization was carried out with calcium ammonium nitrate (270 g kg⁻¹) in three split dosages 40+30+20 kg N/ha (50% level), at the growth stages DC (decimal code) 25/29/51 or 80+60+40 kg N/ha (100% level), at DC 25/29/51 in winter wheat (first year); two split dosages 30+30 kg N/ha at DC 26/33 or 60+60 kg N/ha at DC 26/33 in winter rye (second year); and 60 kg N/ha at DC 31 or 90 kg N/ha at growth stage DC 31 in summer barley (third year).

Phosphorus was applied as Triple Superphosphate (200 g P kg⁻¹) in two levels, (50%) 20 kg/ha and (100%) 39 kg/ha and potassium applied as Korn-Kali® (332 g K kg⁻¹) in two levels (50%) 50 kg/ha and (100%) 100 kg/ha. Both fertilizers (P/K) were applied in spring (to winter wheat on 15.04.2015; winter rye on 15.03.2016 and summer barley on 17.05.2017). In 2014, *Avena sativa* used as a pre-crop received two split dosages of fertilizer, 80 kg N ha⁻¹ at DC 28 (application date 26.03.2014) and 55 kg N ha⁻¹ at DC 31 (application date 30.04.2014). *Zea mays* received 130 kg N ha⁻¹ (as granulated calcium ammonium nitrate based) few days after sowing on 30.04.2014.

3. 3 Plant parameters

3. 3. 1 Density of vegetative tillers and spikes

The density of vegetative tillers and spikes for winter wheat, winter rye and summer barley were determined by counting two times per meter of rows with the help of yardstick for each plot and adjusted to plants per square meter by multiplying the values to the intervals between (12 cm). To avoid the marginal effect the first meters of row from each side was ignored. The average of two-recorded values considered as a representative value.

3. 3. 2 Plant height

Plant height measurement (in cm) was carried out with the help of yardstick considering the distance from the soil surface to the spike apex for five randomly selected plants from each plot. The selection was determined through the plot (42 m²) by ignoring marginal plants to avoid marginal effects. The average from the five replications was considered as a representative value.

3. 3. 3 Leaf area index (LAI)

LAI measurement was carried out by the LAI-2200C plant canopy analyzer from LI-COR[®] Bio-Sciences GmbH (Bad Homburg). The LAI-2200C is equipped with fisheye lens with hemispheric field of view, internal Global Positioning System (GPS) and follows the gap fraction method. LAI-2200C takes advantage of light scattering correction procedure by making K record to calibrate the light sensors and computing the LAI values. Each single measurement encompasses above reading canopy (A reading) and below reading canopy (B reading). The leaf area index (LAI) was measured every two weeks four times per plot to cover the interval (wide distance) between two selective rows. To upload and computing the LAI values the associated software FV2200 from the website of the company.

3. 3. 4 Vegetation index (NDVI/NDRE)

Along with LAI, two different vegetation indices were determined: normalized difference vegetation index (NDVI) and normalized difference red edge (NDRE) by the use of the device RapidScan CS-45 from Holland Scientific[®] (Lincoln, USA) every two weeks and four times per plot. The RapidScan CS-45 incorporates three optical measurement channels. The sensor simultaneously measures crop/soil reflectance at 670 nm, 730 nm and 780 nm (DN: 4501). A unique feature of the RapidSCAN CS-45 sensor is its ability to make height independent spectral reflectance measurements. Plant tissue readily absorbs light in the visible portion of the spectrum (and reflects a small amount typically 2% to 10%) and reflects NIR light (35% to 60%) due to a discontinuity in the refractive indexes between cell walls and intercellular air gaps.

The interplay between canopy reflectance in the visible and NIR portions of the spectrum can be utilized to determine the approximate biomass (or comparative status) of a plant remotely. The RapidSCAN CS-45 has been designed precisely to take advantage of this phenomenon. When near infrared and visible light emitted from the sensor is directed toward a plant canopy, a portion of that light is reflected back to the sensor. The portion of emitted light reflected back to the sensor is detected by an array of spectrally sensitive photosensors. It should be taken into account that the NDVI does not represent a selective value, but rather is to be understood as an image of the photosynthetic performance of the entire vegetation, including the weeds. Furthermore, no conclusions can be drawn about the distinction between sick, less green plants and low vegetation cover (Biewer et al., 2006). The NDVI is calculated from reflection values of the near infrared range (NIR) and the visible red range (red) and can be represented mathematically as follows:

Equation 1:
$$NDVI = \frac{NIR-Red}{NIR+Red}$$

3. 3. 5 Chlorophyll content of leaves (SPAD)

The chlorophyll content of the crop leaves was measured as SPAD (Soil Plant Analysis Development) values with chlorophyll meter SPAD 502Plus (Konica-Minolta®). For that, the youngest leaf (flag leaf) was measured 10 times per plot. The average from the ten replications was considered as a representative value. The values measured by the Chlorophyll meter SPAD-502Plus correspond to the amount of chlorophyll present in the plant leaf. The values are calculated based on the amount of light transmitted by the leaf in two wavelength regions in which the absorbance of chlorophyll is different. SPAD values help to improve crop quality and increase crop yield by providing an indication of the amount of chlorophyll present in the plant leaves.

The spectral absorbance of chlorophyll indicating peaks in the blue (400-500 nm) and red (600-700 nm) regions, with no transmittance in the near-infrared region. To take advantage of this characteristic of chlorophyll, the SPAD-502 measures the absorbance of the leaf in the red and near-infrared regions. Using these two transmittances, the meter calculates a numerical SPAD value, which is proportional to the amount of chlorophyll present in the leaf.

3. 3. 6 Biomass yield

Biomass yield consists of grain as well as straw yield of the target crop. To record the biomass yield, in total eighty test plots with a size of 42 m² were harvested with the cereal plot harvester WINTERSTEIGER. The straw was collected simultaneously with an own constructed tool (load cell model 614-G-100 kg and strain gauge weighting indicator VT100 from Soemer). The harvest took place at the full ripening stage (DC 89) of the cereal crops. The biomass yield (grain plus straw) converted into area yields (dt ha⁻¹) and adjusted to 100% dry matter. Dry matter was determined at 105 °C in a drying cabinet (WTB Binder ED 53) as follow:

Equation 2:

$$DM\ 60\ ^\circ C\ [\%] = \frac{\text{dry weight by } 60\ ^\circ C\ [g] \times 100}{\text{fresh weight } [g]}$$

Equation 3:

$$DM\ 105\ ^\circ C\ [\%] = \frac{(\text{dry weight by } 60\ ^\circ C\ [g] - \text{dry weight by } 105\ ^\circ C\ [g]) \times 100}{\text{dry weight by } 60\ ^\circ C\ [g] - \text{weight of empty shell } [g]}$$

The corrected dry matter (DM corr.) was determined using the following calculation:

Equation 4:

$$DM = \frac{TS\ 105\ ^\circ C \times 100}{TS\ 60\ ^\circ C}$$

Biomass yields of preceding crops

In 2018, the biomass yield (grain and straw) of preceding crops (crimson clover, field bean, oat and maize) were determined. Accordingly, the higher biomass yield of crimson clover (71.3 dt/ha DM 100%) achieved by PK 50%. Field bean achieved the higher biomass yield of 25.6 dt/ha DM 100% by PK+N 50%. In addition, PK+N 50% increased the biomass yield of oat to the maximum level of 174.4 dt/ha DM 100%. The optimal dosage of mineral fertilization (NPK

100%) increased the biomass yield of maize to the maximum level of 143 dt/ha DM 100% (table A.1).

3. 3. 7 Near infrared spectroscopy (NIRS)

The near infrared spectroscopy is an instrumental technique based on measuring the intensity of reflectance or intensity of transmission of radiation from the near infrared region of the electromagnetic spectrum (800-2500 nm) by the test sample. The intensity of the reflection and transmission depends on the rate of absorption of radiation by the sample, which leads to excitation of hydrogen bonds (CH, NH, OH). As the tested samples are very complex in composition, it happens that on the same wavelength, several organic bonds involving hydrogen vibrate producing overlapped spectral bands. Therefore, the resulting NIR spectrum looks like a slightly wavy line with no clearly defined features, with very broad and overlapped molecular overtone and combination bands, which complicate to assign them to specific chemical constituent and make impossible to determine the direct relationship between the concentration of ingredients of interest and the absorbed radiation energy (Pojić et al., 2012).

The NIRS promoted for prediction of more complex constituents such as complex carbohydrates and functional properties of cereal grains. Application of the NIRS technique in wheat quality control has been characterized by rapid development from prediction of major constituents in wheat grains (moisture, protein, oil starch, cellulose) to prediction of functional properties of wheat. The samples used in this study originate from twenty treatments (including 80 plots) and were representative of each plot. More than 700 g grains used for this measurement. Near Infrared Spectroscopy (Perten Inframatic 9500) was used for detecting grain quality parameters.

3. 3. 8 Thousand grain weight (TGW)

Thousand grain weight (TGW) is the weight (in g) of 1000 cleaned seeds from a random sample (Proctor and Rowley, 1983). The Contador seed counter (Pfeuffer) was used to count 100 qualified randomly selected seeds. The weight of the hundred grains was determined by means of analytical balance (KERN EW 420-3NM +/- 0.05 g, Kern und Sohn) in grams. This analysis was repeated three times. The mean value was calculated and converted to a thousand grains. If the determined values were more than three percent apart, the measurement was repeated to decline the variations. Grains should be sorted with a water content of less than 14%.

3. 3. 9 Nitrogen use efficiency (NUE)

Many methods exist with which the NUE of crops can be calculated and evaluated. In own investigated LTE, a method was chosen in which the amounts of N available for the plants (in grain and straw) were taken into account. Therefore, the N use efficiency (NUE_{crop}) is defined as grain productivity per unit N fertilized (equation 5). In addition, the N_{min} value of the soil was analysed in spring at the beginning of the growing season to determine the budget-base nitrogen use efficiency (NUE_{soil}) in the cropping system (equation 6).

Equation 5:

$$NUE_{crop} = \text{Grain yield [kg Grain ha}^{-1}] \times \text{Grain N [kg N kg Grain}^{-1}] / \text{The amount of N fertilized [kg N ha}^{-1}]$$

Equation 6:

$$NUE_{soil} [\text{kg Biomass kg N}^{-1}] = \text{Biomass yield [kg ha}^{-1}] / (\text{The amount of N fertilized [kg N ha}^{-1}] + \text{soil mineral N 0-90 cm [kg N ha}^{-1}])$$

3. 3. 10 NPK analysis of plant samples

Grain (500 g) and straw (200 g) samples randomly selected from each plot (in total eighty plots) at experimental station Giessen for the nutrient and grain quality analysis. Samples grinded finely by Foss grinder CT 193 Cyclotec™ and dried at 105 °C in a drying cabinet (WTB Binder ED 53) for 24 hours for analysis.

Nitrogen (N) analysis

Grinded samples were broken down into pieces with the help of Retsch mixer mill MM 400 to make the samples homogenic for the N measurement. The disruption process with this device carried out with 30 Hz frequency and time set of 3 minutes for each sample. Total nitrogen determination followed by Association of German Agricultural Analytic and Research Institutes “VDLUFA” (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten) methods (Dumas method) through combustion procedure. As a rule of thumb, a particular volume of the samples (grain or straw) used for N measurement. This volume is determined by Elementar-Unicube C/N analyser by testing samples.

In this measurement nitrogen is converted into nitrogen monoxide (NO) at temperatures of above 620 °C by oxygen absorption from all forms of bonding. These are mitigated to elemental nitrogen (N₂) by metallic copper; the amount of N₂ is then quantified. Before running the measurement with Unicube, device must be calibrated by chemicals like sulphanilamide 2 mg (± 0.0001) and wheat standard (niva - 33802156) of 10 mg. The peak areas measured by the Unicube are converted to the N content using the following formula; F is a factor, which is required for the conversion to the N content.

Equation 7:

$$F = (\text{Weight of standard [mg]} \times \text{N content of standard [\%]}) / (\text{Peak unit})$$

Sample volume depending to the quality of the material ranges between 5 to 10 mg to reach the basic peak (1200 unit) by the instrument. To this approach samples weighted into special aluminium foil provided by Elementar Company by using a fine scale (Mettler Toledo-XP 105). Every single sample had a replication control and their repeatability ratio adjusted to less than 5%.

3. 3. 11 Potassium (K) and phosphorus (P) analysis

P and K analysis was accomplished by preparing ashes with incineration procedure of one-gram plant (grain or straw) materials. Samples were weighted in incinerating porcelain dishes and put in Muffle furnace (Nabertherm N 11/HR) at 550 °C. The incineration procedure

of Muffle furnace follows a few times sets with different heating sequences starting at 125 °C, 250 °C and 550 °C, which needs 12 hours for preparing ashes.

Potassium analysis followed by “VDLUFA” method. After incineration procedure, ashes rinsed with 5 ml of nitric acid (HNO₃) 20% (HNO₃ 20%: 333 ml of nitric acid to the volume of 1000 ml distilled water) by heating the samples on the Lab heater. The extraction followed by filtering the samples (plant materials) with the filter paper number 615 (grade Macherey-Nadel 615 ¼, 600 mm). After filtrations, samples are adjusted to the volume of 100 ml. Every single sample had a replication control and their repeatability ratio adjusted to less than 5%.

Accordingly, after filtration, flame photometer (BWB-XP) used for K analysis. In this method, solution was sucked in by Flame photometer and sprayed into a propane air flame. The salts are atomized by thermal dissociation. Due to the thermal excitation, the resulting atoms are able to emit light with a characteristic wavelength. This wavelength for K set as 766 nm. The emission of the elements is thus measured. In the beginning of the measurement, a calibration curve was depicted by using the calibration solutions (table 3). The measured values were given in ppm or mg/l. The final values of nutrient uptake adjusted in kg per hectare by using the dry matter.

Table 3: Concentrations of the K contained in the calibration solutions.

ppm	E.C. 0	E.C. I	E.C. II	E.C. 2.5	E.C. III	E.C. IV	E.C. V	E.C. VI
K	0	20	40	60	80	120	160	200
The volumes required for calibration solutions								
ml/250 ml	0	2.5	5	7.5	10	15	20	25

Phosphorous analysis followed by “VDLUFA” method. After finishing filtration (the same like K analysis), the measurement performed colorimetrically, and the light absorption measured with the spectrophotometer (Specord 205, Analytik Jena AG). To this approach VM reagent stock solution 1:1:1 used for P determination. VM reagent stock solution encompasses of ammonium monovanadate (2.5 g into the volume of one liter by using distilled water), ammonium heptamolybdate (50 g in 1-liter distilled water) and 1- liter nitric acid (HNO₃). Then VM reagent stock solution diluted (to 400 ml into the volume of 1 liter). The analysis accomplishing by using 10 ml of sample plus 30 ml of diluted VM reagent stock solution. The absorbance was then measured at the wavelength of 436 nm. In the beginning of the measurement, a calibration curve was depicted by using the calibration solutions (table 4). The measured values were given in ppm or mg/l. The final values of nutrient uptake adjusted in kg per hectare by using the dry matter.

Table 4: Concentrations of the P contained in the calibration solutions.

ppm	E.C. 0	E.C. I	E.C. II	E.C. 2.5	E.C. III	E.C. IV	E.C. V	E.C. VI
P	0	6	12	24	36	48	72	96
The volumes required for calibration solutions								
ml/250 ml	0	1	2.5	5	7.5	10	15	20

3. 4 Soil Parameters

In LTE “BNF” several chemical and biological investigations were implemented extensively and regularly each year. Total Carbon (Ct), total nitrogen (Nt), nitrate $\text{NO}_3^- \text{N}$, water soluble carbon (DOC), water soluble nitrogen (DON), total organic carbon (TOC), total nitrogen bound (TNb), microbial biomass carbon (SMB C) were the frequent measurement from 2015 to 2020. In addition, some more soil analysis like pH value, ammonium ($\text{NH}_4^+ \text{N}$), bait strip investigation, seasonal CO_2 respiration of the soil and mesofauna abundance were carried out.

For soil chemical ($\text{NO}_3^- \text{N}$, $\text{NH}_4^+ \text{N}$, Ct, Nt, DOC, DON, TOC, TNb) and biological analysis (SMB C) 300 g samples are taken by hand or machine from each plot (80 plots) and through the soil profile (0-30, 30-60, 60-90 cm). Samples directly were frozen in minus 20 °C in the experimental station Giessen (figure 4 & 5).



Figure 4 (left): Soil sampling techniques, steel auger for drilling and taking soil sample (Photo: Yavar Vaziritabar). **Figure 5 (right):** Sampling device (Multiprob 120-UP)-Nietfeld company, Experimental station “Weilburger Grenze” Giessen (Photo: Markus Kolmer).

3. 4. 1 Nitrate (NO_3^-) N analysis

Nitrate (NO_3^-) N analysis was carried out by taking soil samples at the beginning of growing stage in spring and/or three weeks after harvest of winter wheat, winter rye or summer barley. Four samples were taken from the middle of each plot (300 g) through the whole soil profile (0-30, 30-60 & 60-90 cm). In the initial step of nitrate analysis soil samples were sieved through 5 mm sieve. Each sample has a particular label, which include technical information including plot number, treatments detail (pre-crop or levels of NPK fertilization) and the depth of the soil.

Nitrate (NO_3^-) N analysis carried out based on calcium chloride extraction, a standard method from VDLUFA. In this analysis 50 g field moist soil weighted into shaking flasks. In the next step, 250 ml extraction solution (9.19 g calcium chloride dihydrate into volume of 5000 ml using distilled water - CaCl_2 0.0125 mol/l) added to the shaking flasks. Then the samples were shaken with 200 rpm about 1 hour and 30 minutes by using lab shaker Gerhardt. After shaking, the samples were filtered off by using filter paper number 619 (grade Macherey-Nadel 619 ¼, 600 mm) and the first drops of the filtrate were discarded (figure 6).

For the analysis, in one section, 25 ml of the sample solution were pipetting in two-test glass tubes (either as a sample or as a reference) and 1 ml of sulfuric acid 10% (55 ml in 1 liter distilled water) was added. The samples were mixed appropriately with the lab tube shaker and three to four zinc granules were added to the reference tube serving to activate zinc granules with sulfuric acid for nitrate reduction in reference tube. The samples are left over the night with the stopper loosely attached. Measurement performed with the UV/VIS spectrophotometer Specord 205, Analytik Jena AG.



Figure 6: Soil extraction and filtration method for nitrate (NO_3^-) N analysis, laboratory of the department of Agronomy (photo: Yavar Vaziritabar).

Standard calibrations were carried out in two sequences. First preparing stock standard solution by Natrium-nitrate (3.0341 g into volume of 500 ml) and the next is standard calibration, which requires 100 ml of stock standard solution into 1-liter volume. Finally, the calibration solution devoted by 10 ml of standard calibration mixed with CaCl_2 extraction solution (CaCl_2 0.0125 mol/l) into 500 ml volume. Then series of standard calibrations from calibration solution should be set up for implementing the measurement (table 5).

Table 5: Concentrations of the nitrate (NO_3^-) N ($\mu\text{g}/25$ ml) contained in the calibration solutions.

ppm	E.C. 0	E.C. I	E.C. II	E.C. III	E.C. IV	E.C. V
(NO_3^-) N $\mu\text{g}/25$ ml	0	5	10	20	30	40
The volumes required for calibration solutions						
ml/25 ml	0	2.5	5	10	15	20

The measuring device was calibrated with the standard series at 210 nm wavelength in which both glass tubes (sample and reference tubes) could be read off simultaneously in parallel. The software programming with linear equation, number and concentration of standard calibrations. Ultimately, in order to determine the content of nitrate (NO_3^-) N in unite

of kg ha⁻¹ the value of the water content is required (Equation 8 & 9). So that the dry mass of the soil samples was calculating by drying 50 g soil in drying cabinet (WTB Binder ED 53) at 105 °C for 24 hours.

To make the analysis homogenic and reducing the proportion of error all the process for soil extraction and measurement run professionally and simultaneously for 160 samples through each depth. The variability of each sample with its replication adjusted below 5%.

Equation 8 [mg Nitrate-N/100 g soil]:

$$(NO_3^-)N = \mu g N / 25 ml (250 ml \times 0.1) / (50 [g] \times 25 [ml])$$

Equation 8 is required to convert the (NO₃⁻) N value to mg Nitrate-N per 100 g soil.

Equation 9 [kg Nitrate-N/ha]:

$$\text{Nitrate per kg/ha} = ((NO_3^-)N \times \text{soil depth} \times \text{soil density}) / (\text{dry mass}) \times 100$$

Equation 9 is required to convert the (NO₃⁻) N value to kg ha⁻¹.

3. 4. 2 Total carbon (Ct) and total nitrogen (Nt) analysis

According to VDLUFA method, the Ct and Nt contents of the soil (0-30, 30-60 & 60-90 cm) were determined by drying 100 g soil at 40 °C (in a drying cabinet - WTB Binder ED 53) for 3 days. After that the soil was sieved into 2 mm by using an analytic lab sieve. To make the soil homogenic, samples were broken down with the help of Retsch mixer mill MM 400 to the soil particles. The disruption process of the soil with this device carried out with 30 Hz frequency and 4 minutes for each sample.

Ct and Nt determination of the soil were done according to the Association of German Agricultural Analytic and Research Institutes "VDLUFA" (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten) methods ("Dumas" method) through combustion procedure. As a rule of thumb, a particular volume of the soil samples used for C and N measurement. This volume will determine by Elementar-Unicube C/N analyser by testing samples.

Before running the measurement with Unicube, the device must be calibrated by chemicals like sulphanilamide 2 mg (± 0.0001) and soil standard (niva - 33802182) of 35 mg. The peak areas measured by the Unicube are converted to the N content using the following formula; F is a factor which is required for the conversion to the N content (Equation 7).

Sample volume depending to depth (0-30, 30-60 and 60-90 cm) and the quality of the soil ranges between 35 to 40 mg to reach the basic peak (1200 unit) by the instrument. To this approach samples weighted into special aluminium foil provided by Elementar Company by using a fine scale (Mettler Toledo-XP 105). Every single sample had a replication control and their repeatability ratio adjusted to less than 5%.

In this method through the oxidative combustion at 950 - 1000 °C approximately, the digestion of the soil substances occurred. The gas mixture, produced during combustion is separated according to its components and recorded by Unicube.

3. 4. 3 Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) analysis

To determine DOC and DON of the soil (0-30, 30-60 & 60-90 cm), samples were dried at 40 °C (in a drying cabinet - WTB Binder ED 53) for 3 days. Then the soil was sieved into 2 mm by using an analytic lab sieve (figure 25) and 25 g of the prepared soil were weighted into shaker flasks and 125 ml calcium chloride (CaCl₂) were added to the flask and shaken for 1 hour and 30 minutes. The samples were then filtrated by using filter paper number 619 (grade Macherey-Nadel 619 ¼, 600 mm) and the first drops of the filtrate were discarded. The samples were then storage into 100 ml plastic bottles and frozen to minus 20 °C for the analysis.

The analysis carried out with the autoanalyzer device and followed the VDLUFA method (1997, chapter A 6.1.7.0). The measurement takes advantage of photometric method using UV lights. DON concentrations are estimated from different N fractions (including Nt, NH₄⁺N & NO₃⁻N) in 540 nm wavelength and detecting by sulfanilamide (2.5 g), N-1-Naphtyl-ethylendiamindihydrochlorid (0.125 g) and phosphoric acid (40 ml) in a yellow or phosphorous color. DON is calculated from a multi-section measurement of Nt, NH₄⁺N & NO₃⁻N and calculating as follow (DON [mg N/L] = TN [mg N/l] - NO₃⁻N [mg N/L] - NH₄⁺N [mg N/L]), which needs 24 minutes for analyzing a sample. The measurement has three peaks, (NO₃⁻) N (first peak takes around 7 minutes), (NH₄⁺) N (second peak takes around 14 minutes) and Nt (the third peak takes around 24 minutes).

DOC determination performs by oxidation path by potassium peroxide sulfate (K₂S₂O₈). So that the water-soluble organic carbon transform into CO₂ and passed through the membrane path by Autoanalyzer and reducing by phenolphthalein solution and takes the pink color. UV detector takes the solution and read the value at 550 nm wavelength. Every single sample had a replication control and their repeatability ratio adjusted to less than 5%.

To do this, series of standard calibrations from calibration solution should be set up for implementing the measurement (table 6).

Table 6: Concentrations of the N fractions and DOC contained in the calibration solutions.

Standard solution	E.C. 0	E.C. I	E.C. II	E.C. III	E.C. IV
(NO ₃ ⁻) N (mg N/l)	0.25	0.5	1	2	3
(NH ₄ ⁺) N (mg N/l)	0.12	0.25	0.5	1	1.5
Nt (mg N/l)	0.37	0.75	1.5	3	4.5
The volumes required for calibration solutions					
DOC (ml/100 ml)	1	2.5	3.75	5	–

3. 4. 4 Total organic carbon (TOC) and total nitrogen bound (TNb)

To determine TOC and TNb of the soil in 0-30, 30-60 and 60-90 cm, soil samples were dried at 40 °C (drying cabinet WTB Binder ED 53) for 3 days. After them the soil was sieved

into 2 mm by using an analytic lab sieve (figure 10). 25 g of the prepared soil were weighted into shaker flasks and 125 ml calcium chloride (CaCl_2) were added to the flask and shaken for 1 hour and 30 minutes. The samples were then filtrated by using filter paper number 619 (grade Macherey-Nadel 619 $\frac{1}{4}$, 600 mm) and the first drops of the filtrate were discarded. The samples were then storage into 100 ml plastic bottles and frozen to minus 20 °C for the analysis.

The analysis was carried out with Vario TOC cube (Elementar-TOC/TNb analyzer). The measurement follows the standard principle of DIN EN 1484. In this method the oxidation of bound carbon as a result of high temperature digestion and catalytic post combustion is achieved in 847 °C. Any formed CO_2 concentrations are easily purified through a halogen trap that is equipped with a subsequent 3-step intensive gas drying system. However, to detect these CO_2 concentrations, a wide range NDIR-photometer is utilized. In addition, the determination of bound total nitrogen (TNb) as NO can also be achieved with the use of an optional electrochemical sensor (EC), infrared- (NDIR) or chemiluminescence detector (CLD) down to ppb level DIN EN 12260. Every single sample had a replication control and their repeatability ratio adjusted to less than 5%.

3. 4. 5 Cation exchange capacity (CEC)

The cation exchange capacity (CEC) of the soil (0-30 cm) was performed based on Mehlich method DIN 19684-8 (1938). In this method, barium (Ba) was determined by losing the soil cation bounds and occupying the exchange places of the soil cations. The exchanged barium is then replaced with a magnesium chloride solution in the soil solution. Five different working solutions are required for the extraction solutions. A buffer made from triethanolamine and barium chloride that is adjusted to pH 8.1, 0.2 n BaCl_2 , 0.02 n BaCl_2 , distilled water and 0.2 n MgCl_2 .

First, 5 g soil is weighed into a 250 ml flask with a funnel covered with a blue band filter. To improve the method to have less disruption during pipetting, one extra blue band filter on the top of each funnel was added. It is then filtered five times with 15 mL of the buffer, washed three times with 10 ml of 0.2 n BaCl_2 , once with 5 ml of 0.02 n BaCl_2 and then washed three times with distilled water.

To catch the barium, new 250 ml flasks are placed under the soil solution and washed through nine times with 25 ml of 0.2 n MgCl_2 and once with 21 ml. The flasks were adjusted to the volume of 250 ml with MgCl_2 . The subsequent washout may only take place when the previously supplied liquid has overflowed into the flask. To avoid mistakes, the liquid should be added slowly and not directly to the soil sample. A pipette with a pole ball can be used for this purpose, for example. In addition, if the injection pump was used and the liquid was immediately added to the sample, after several runs a small indentation formed at the point where the liquid was added, for that reason I recommend the establishment of blue band filter paper on the top of funnel.

In addition, the soil particles were repeatedly mixed, which led to the structure being disturbed and the sample becoming finer as a result. The liquid ultimately flows more slowly through the swirled floor. Barium was measured by diluting samples to the ratio of 1:10 and

using the atomic adsorption spectrometer "SpectrAA 220 FS" Measured at 553.6 nm. Other cations like Ca and Mg also measured by atomic adsorption spectrometer "Perkin Elmer 3100" measured at 422 and 285 nm respectively. Finally, potassium (K) analyzed by Flame photometer BWB-XP.

The following calculation used for CEC determination of the soil. Every single sample had a replication control and their repeatability ratio adjusted to less than 5%.

Equation 10:

$$cmolc (kg\ soil)^{-1} = \frac{(measured\ value - blank\ value) \times 10 \times 0.25\ l \times 100\ g\ soil \times 2\ mmol}{137.33\ g\ mol^{-1} \times 5\ g\ soil}$$

3. 4. 6 Microbial biomass carbon of the soil (SMB C)

SMB C analysis of the soil (0-30 cm) was performed according to Vance et al. (1987) by sieving 100 g fresh soil into 2 mm (using analytic lab sieve), and they were cleaned from plant residues or stones. The analysis followed by weighting 12.5 g soil in two sections fumigated and non-fumigated samples. In fumigated section, samples weighted accurately (12.5 g) in glass shells and kept in a desiccator. So that each desiccator filled with wet papers and a Becher of soda lime.

Each desiccator contains maximum eight glass shells, four additional Becher's which were filled with boiling stones, and 25 ml chloroform (ethanol free). By fumigated section, a thick layer of silicon grease used to seal the desiccator by evacuating procedure with the pump (2 minutes evacuation after boiling chloroform). Then the desiccator was kept under fume cupboard at 25 °C in a dark condition for 24 hours.

Besides, non-fumigated section was also performed in parallel by weighting 12.5 g fresh soil into shaking flasks. For both sections (fumigated and non-fumigated samples) 50 ml of potassium sulfate solution (K₂SO₄, 0.5 mol) used for extraction. The extraction was carried out with standard lab shaker "Gerhardt" about 200 rpm for 30 minutes; and the filter paper number 619 (grade Macherey-Nadel 619 ¼, 600 mm) used for this analysis.

The measurement carried out with UV photometer (L003) at 280 nm wavelength and using 1 cm Quarz-Cuvette from glass. The difference between fumigated and non-fumigated samples results in increasing of UV absorbance. Every single sample had a replication control and their repeatability ratio adjusted to less than 5%. Ultimately, the dry mass of soil was calculated by weighting 5 g soil and drying at 105 °C in a drying cabinet (WTB Binder ED 53) for one day. The following equations used for SMB C calculations for both sections (fumigated and non-fumigated samples).

Equation 11:

$$Soil\ dry\ matter\ (g) = \frac{weighted\ sample \times dry\ matter\ [%]}{100}$$

Equation 12:

$$Ext./g\ dw = \frac{Extinction\ value \times dillution\ factor}{soil\ dry\ matter}$$

Equation 13:

$$SMB C = (Ext./g dw fumigated - Ext./g dw non - fumigated) \times 34735^*$$

* 34735 used as a slope value for UV photometer.

3. 4. 7 Soil fauna feeding activity based on bait strips

Bait strips or bait lamina test is a soil biological investigation indicating soil ecosystem (species composition) and typical soil functional parameters like turnover processes of the soil organic matter. Bait strips are an integrative method, since it assesses both soil microbial and soil invertebrate activity in the investigated soil layers and do not allow for a differentiation in the role played by different organism groups (Kratz, 1998). The patent of bait lamina test system is hold by Terra Protecta GmbH, Berlin, Germany consisting of perforated PVC stripes (16 pierced holes) filled with the standard substrate mixture (cellulose, bran flakes, active coal). Bait strips consist of plastic strips 120 x 6 x 1 mm in size, which have a pointed tip at the lower end. In the lower part (85 mm) of each strip, 16 holes of 1.5 mm diameter are drilled with a 5.5 mm spacing. Bait strips should have enough consistency, elasticity and stability to be placed easily in moist, fine and coarse soil without damage. The bait strips used in LTE "BNF" were prepared and provided by Landesbetrieb Hessisches Landeslabor (LHL) (Schloss Eichhof, 26251 Bad Hersfeld, Germany).

It is assumed that the disappearance of the bait material is directly associated to the feeding activity of soil invertebrates, even if microbial processes may play a minor role. This investigation carried out by planting 21 bait strips in selected plots (with the area of 42 m²). Bait strips settled in the middle if the plots in three rows and the interval between individual strips adjusted to 10 cm. Crimson clover, oat and fallow land (factor A); NPK 100% and control (factor B) within four replications were chosen for the settlement of the bait strips.

Also, for each plot one strip was removed instantly as a control. The investigation covered the period of 20 days, and carried out two months after harvesting of winter rye (on 05.10.2020) when the rye stubbles were in the soil. Then stripes were collected and washed out carefully with distilled water in the laboratory to exclude the soils. Stripes were wrapped in the plastic bags and stored for a week in the cool room at 5 °C. The observations of eaten bait patches on each strip (embedded in 16 holes) counted and converted to the percentage.

3. 4. 8 Mesofauna abundance

Soil mesofauna abundance was used as soil quality indicator. The investigation was carried out by taking randomly five samples from the middle of each plot. Samples are taken in August 2020 with the help of soil cores (of 5 cm diameter) to the depth of 15 cm (Macfadyen, 1961) two weeks after harvesting winter rye.

After taking soil samples they were kept into the cool box and immediately transported to the laboratory for some rudimentary analysis like determining the weight, height and the volume of the samples, each sample is labelled base on the plot number and relevant treatment details. Samples took place up to the down side into the sieve in order the soil fauna

can easily evacuated; and sample attached to the sieve settled on a container, which was filled (5 ml) with ethylene glycol.

The analysis followed the improved funnel-type extractors (Macfadyen, 1961). Samples were settled in a container with halogen lamps for two weeks using high gradient technique for soil extraction. In this method samples were confronted with different range of temperature in 14 days. The basic temperature starts at 20 °C so that it increases every day about 2 °C to reach to the maximum level of 60 °C in a few least days (figure 7).



Figure 7: Drying chamber and sampling method to extract soil arthropods, high gradient technique, LTE-BNF (photo: Yavar Vaziritabar).

3. 4. 9 Soil CO₂ efflux measurement

Soil CO₂ efflux measurement carried out with a portable smart survey chamber (8200-01S Licor, Lincoln, NE USA) accompanying with Li-cor 870 CO₂/H₂O infrared gas analyser-IRGA (SSA-1005). The closed-chamber method is the most common approach used to estimate the fluxes of CO₂ (F_c , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and other trace gases at the soil surface. In this method, the air flow from a chamber pumped and circulated to an infrared gas analyzer (IRGA) and then sent back to the chamber (figure 8). F_c is estimated with chamber volume, soil surface area, air temperature, atmospheric pressure, and the rate of CO₂ concentration increase inside the chamber (dC_c/dt , $\mu\text{mol mol}^{-1} \text{s}^{-1}$) which has been on the soil surface for a short period of time (equation 14).

Equation 14:

$$F_c = \frac{PV}{RTS} \frac{dc_c}{dt}$$

Where P is the atmospheric pressure (Pa), V (m³) is the total system volume, including the volume of the chamber, the pump, and tubing in the measurement loop, R is the gas constant (8.314 Pa m³ °K⁻¹ mol⁻¹), T is the absolute temperature (°K), and S (m²) is the soil area covered by the chamber (Madsen et al., 2009).



Figure 8: Soil CO₂ efflux measurement by Licor-870 CO₂/H₂O infrared gas analyser-IRGA (SSA-1005) in LTE (photo: Yavar Vaziritabar).

The homemade soil collars constructed from thick-walled (8-inch) SDR-41 PVC pipe according to Licor criteria. Collar dimensions followed as 20.3 cm inside diameter, 21.3 cm outside diameter and approximately 11 cm height. To bevel the bottom edge to an angle of 24° a grinder or coarse file is used (figure 9).



Figure 9: Chamber-collar offset settlement for soil CO₂ efflux measurement, LTE-BNF (photo: Yavar Vaziritabar).

Depending on plot size, four or five rings were used and distributed within each plot. Rings suitably settled and adjusted to 5 cm (collar offset) of the soil surface though the bare soil and if necessary, cleaned from crops or plant residues. As Li-COR analyser integrated pressure

and temperature-controlled optical component as well as GPS logging (smart chamber), it takes 10 to 15 minutes to warm up and stabilize the optics bench prior to taking measurement. In the smart chamber software, the stabilization indicating as a green dot in the upper-left portion. Measurement programming by collar offset (5 cm), soil area (318 cm²), IRGA(s) volume (80.46 cm³), chamber volume (4244.10 cm³) and the total volume (5914.56 cm³). The total volume is estimated with equation 15.

Equation 15:

$$\text{Total volume} = (\text{Offset} \times \text{Soil Area}) + \text{IRGA}(s) + \text{Chamber}$$

Measurement cycles by observation length of three minutes (180 seconds), dead-band of 25 seconds and post purge of 45 seconds. The exponential approach of CO₂ concentration over the time series reached by the maximum numbers of iteration and followed by a comparison of the slopes from the linear regression and the exponential regression (Madsen et al., 2009).

Environmental measurements

The Steven HydraProbe soil sensor (Campbell Scientific®) implemented simultaneously along with smart chamber to detect the most important soil parameters like: soil temperature (°C), soil moisture (m³ m⁻³), electrical conductivity (S m⁻¹) and dielectric permittivity. The Stevens probe has four Tines with detecting depth of 45 mm. In addition, experimental station in GI equipped with weather station, which provide temperature (20 cm and 2 m from the soil surface), and precipitation individually. Smart chamber is also capable to detect the temperature inside the chamber during the measurement (figure 10).



Figure 10: The Stevens HydraProbe soil sensor (photo: Yavar Vaziritabar).

Data processing and statistical analyses

Soil Flux Pro™ Software was used to merge the data file (“json” format) achieved by Li-870. The exponential flux rate values were calculated by the average of four or five individual rings for each plot.

3. 5 Statistical analyses

Treatment factors (pre-crop and NPK fertilization) on plant and soil parameters were tested using multi-factor analysis of variance based on GLM (Generalized Linear Model) procedure. To this approach two-way ANOVA model based on the two test factors in LTE “BNF” was used to examine the difference of different categorical independent variables on one dependent variable as well as the interaction between both test factors (pre-crop and NPK fertilization). Differences between groups were investigated based on model coefficients and using the Tukey’s post-hoc analysis. The term “significant” was used to describe whether the mean values differed very clearly with a probability (p) of $0.01 < p \leq 0.05$. The standard error (SE) was calculated based on standard deviation. So that, the standard deviation was divided by the square root of the sample size (n).

All statistical analyses were performed using SAS (Version 9.4; SAS Institute Inc.; Cary, North Carolina, USA). The commands that were implemented and used to perform the ANOVA in SAS are shown below.

```
data x;
input preceding_crop fertilization rep variable (a);
cards;

;
proc print;
proc capability normaltest;
var (a);
histogram/normal;
run;
proc glm;
class rep preceding_crop fertilization;
model y=rep preceding_crop fertilization preceding_crop*fertilization/ss3;
means preceding_crop fertilization preceding_crop*fertilization/Tukey alpha=.05;
means preceding_crop fertilization preceding_crop*fertilization/lsd cldiff;
lsmeans preceding_crop*fertilization /slice=preceding_crop tdiff;
run;
```

Microsoft Office used for making graphs and tables in this investigation. The linear regression model was used to calculate the correlation coefficient between CO₂ efflux and soil parameters (air/soil temperature or moisture).

4 Results

4. 1 Effect of preceding crops and mineral NPK fertilization on soil parameters

4. 1. 1 Nitrate (NO₃⁻) N content of the soil

September 2015

The first nitrate test was carried out with soil samples taken in September 2015, three weeks after harvesting winter wheat. Thus, the data represent the residual amounts of N (in 0-40 cm) that were left in the soil after winter wheat. The analysis of variance revealed that neither pre-crops (p -value = 0.068) nor NPK fertilization (p -value = 0.759) has increased the nitrate (NO₃⁻) N content of the topsoil (0-20 cm). Furthermore, there was no significant interaction between pre-crops and mineral fertilization (p -value = 0.851) (table 7). The nitrate amounts were on a low level varying from 10 kg ha⁻¹ to 15 kg ha⁻¹ (data not shown).

In contrast, in the second depth (20-40 cm) a significant effect of preceding crops on nitrate N content of the soil was found (p -value < 0.001). It was observed that crimson clover (41 kg ha⁻¹), field bean (34 kg ha⁻¹) and oat (37 kg ha⁻¹) led to increase the nitrate (NO₃⁻) N content of the soil (20-40 cm) compared to maize (31 kg ha⁻¹) and fallow (27 kg ha⁻¹). On the other hand, neither an effect of NPK fertilization (p -value = 0.656) nor an interaction effect between both factors (p -value = 0.886) was observed (table 7).

Table 7: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO₃⁻) N of the soil after harvesting of winter wheat in September 2015, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Nitrate (NO ₃ ⁻) N content of the soil (kg ha ⁻¹) September 2015		
		0-20 cm	20-40 cm	Sum 0-40 cm
Preceding crop (PC)	Fallow	11.0 ns	27.2 d	38.2 c
	Crimson clover	13.1 ns	41.4 a	54.5 a
	Field bean	13.8 ns	33.8 bc	47.6 ab
	Oat	13.7 ns	37.2 ab	50.9 ab
	Maize	13.9 ns	31.1 cd	45.0 bc
NPK fertilization (NPK)	No fertilization	13.9 ns	35.9 ns	49.8 ns
	PK 50%	13.6 ns	32.9 ns	46.5 ns
	PK+50% N	12.9 ns	33.0 ns	45.9 ns
	PK+100% N	15.0 ns	34.7 ns	49.7 ns
p -value	PC	0.068	< 0.001	< 0.001
	NPK	0.759	0.656	0.587
	PCxNPK	0.851	0.885	0.954
LSD 5% (in kg ha ⁻¹)	PC	3.1	6.0	8.8
	NPK	2.6	5.4	7.8
	PCxNPK	4.4	12.1	17.6

September 2016

The second nitrate analysis of the soil was carried out in September 2016, three weeks after harvesting winter rye and two years after the pre-crops. The analysed data showed that crimson clover (34 kg ha⁻¹), field bean (34 kg ha⁻¹), oat (31 kg ha⁻¹) steady increased the nitrate (NO₃⁻) N of the topsoil (0-30 cm) compared to maize (30 kg ha⁻¹) and fallow (26 kg ha⁻¹). So far, neither an effect by NPK fertilization (p -value = 0.051) nor. In addition, no significant differences between main factors (pre-crops and NPK fertilization) on the nitrate (NO₃⁻) N content of the topsoil were found (p -value = 0.721) (table 8).

In accordance with the result from the topsoil, also in the second depth (30-60 cm) significant differences of nitrate (NO₃⁻) N values caused by preceding crops were found (p -value < 0.001) (table 8). In this depth, crimson clover (55 kg ha⁻¹) and field bean (50 kg ha⁻¹) was increased the nitrate (NO₃⁻) N to the maximum level in the second year of the crop rotation after preceding crops and the lowest (NO₃⁻) N content was achieved by oat (42 kg ha⁻¹) and maize (34 kg ha⁻¹). So far, no significant effect of NPK fertilization was found on (NO₃⁻) N of the soil (30-60 cm) (p -value = 0.123). In addition, there was no significant interaction between main factors (pre-crop and NPK fertilization) on (NO₃⁻) N of the soil layer 30-60 cm (p -value = 0.648) (table 8).

In the deepest soil layer (60-90 cm) that was examined the (NO₃⁻) N level varied from 18.8 to 48.5 kg ha⁻¹ among treatments (table 8 and figure 11). It must be taken into account that there was a significant interaction between both factors (pre-crops and NPK fertilization) indicated by p -value of 0.024 (figure 11). This was caused by higher (NO₃⁻) N values in the variants without NPK and with 100% NPK each after oats and maize.

Table 8: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO₃⁻) N of the soil after harvesting winter rye in September 2016, LTE "BNF" Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Nitrate (NO ₃ ⁻) N content of the soil (kg ha ⁻¹)			
		September 2016			
		0-30 cm	30-60 cm	60-90 cm	Sum 0-90 cm
Preceding crop (PC)	Fallow	26.3 c	44.3 b	22.1 c	92.7 c
	Crimson clover	34.3 a	55.0 a	25.4 bc	114.7 a
	Field bean	34.0 ab	50.2 ab	24.8 bc	109.0 a
	Oat	30.5 ab	42.3 bc	33.7 a	105.2 ab
	Maize	30.3 b	33.8 c	31.6 ab	97.1 bc
NPK fertilization (NPK)	No fertilization	34.1 ns	49.1 ns	30.5 ns	113.7 a
	PK 50%	31.1 ns	47.2 ns	26.2 ns	104.5 b
	PK+50% N	29.8 ns	44.4 ns	22.9 ns	97.1 b
	PK+100% N	29.2 ns	39.9 ns	30.5 ns	99.6 b
p -value	PC	< 0.001	< 0.001	0.008	< 0.001
	NPK	0.051	0.117	0.051	< 0.001
	PCxNPK	0.721	0.602	0.011	< 0.001
LSD 5% (in kg ha ⁻¹)	PC	4.2	8.8	7.1	10.5
	NPK	3.7	7.9	6.4	9.4
	PCxNPK	8.3	17.7	14.2	21.0

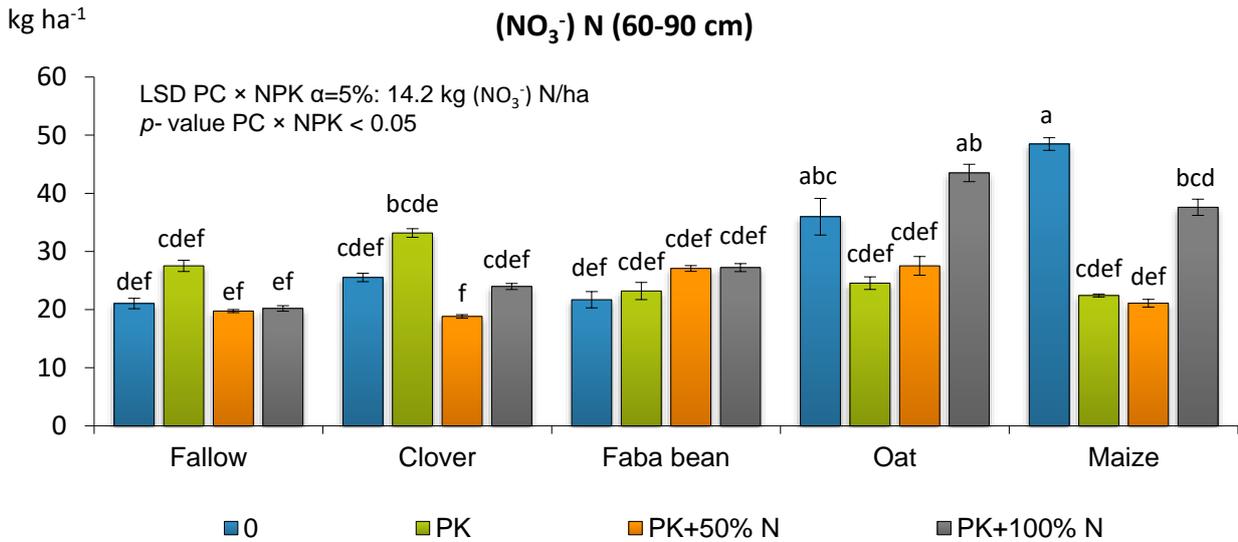


Figure 11: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO₃⁻) N of the subsoil (60-90 cm) (September 2016), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

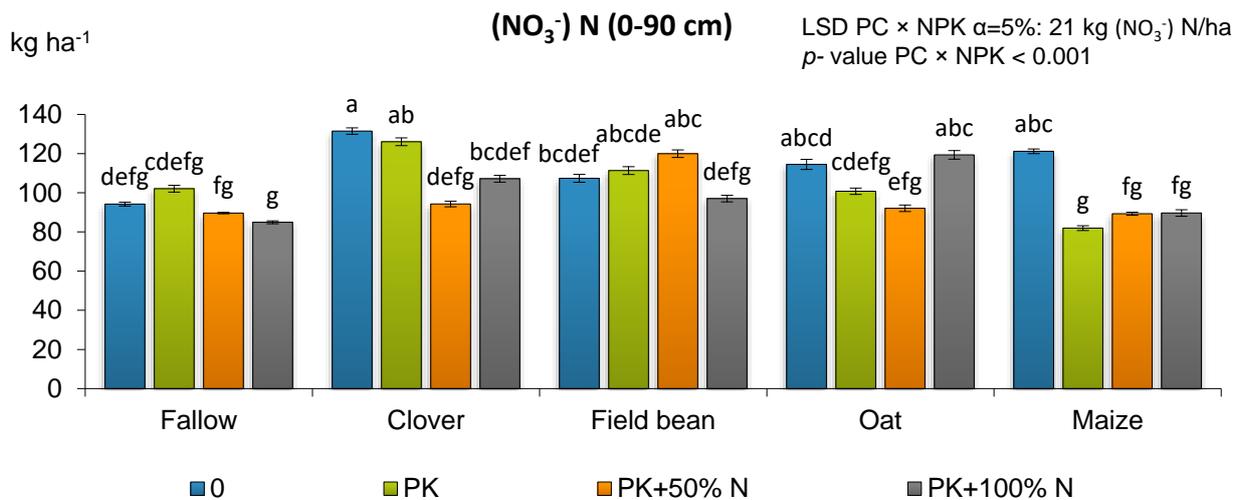


Figure 12: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO₃⁻) N of the subsoil (30-60 cm) (September 2016), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

September 2018

In September 2018, two weeks after harvesting pre-crops, as expected a significant effect of preceding crops on (NO₃⁻) N was found both in first depth 0-30 cm and second depth 30-60 cm as well as in the soil profile 0-60 cm (*p*-value < 0.001 each). Among different preceding crops or land uses, fallow (39 kg ha⁻¹) achieved highest (NO₃⁻) N value compared to crimson clover (28.6 kg ha⁻¹), field bean (24 kg ha⁻¹), oat (21 kg ha⁻¹) and maize (22 kg ha⁻¹).

So far, no significant differences were found by NPK fertilization (p -value = 0.383). The (NO_3^-) N level of the topsoil recorded from 25 to 29 kg ha^{-1} by mineral fertilization. In addition, no significant effects between the main factors (pre-crops and NPK fertilization) were found (p -value = 0.375). The (NO_3^-) N level of the topsoil varied at 15 to 46 kg ha^{-1} by the treatments (pre-crops and NPK fertilization) (table 9).

In subsoil (30-60 cm), significant effect of pre-crops on (NO_3^-) N of the subsoil (30-60 cm) was found (p -value < 0.001). Therefore, fallow (10 kg ha^{-1}), clover (7 kg ha^{-1}) and field bean (6 kg ha^{-1}) achieved the higher levels of (NO_3^-) N in subsoil compared to oat (2 kg ha^{-1}) and maize (3 kg ha^{-1}). Furthermore, no significant effects of NPK fertilization were found on (NO_3^-) N of the subsoil (30-60 cm) (p -value = 0.217). More to that significant interaction effect between pre-crops and NPK fertilization were found (p -value < 0.001). The (NO_3^-) N level of the subsoil (30-60 cm) varied from 1 to 16 kg ha^{-1} (table 9 and figure 13).

In addition, through the soil profile (0-60 cm), the higher amount of nitrate (NO_3^-) N was found after fallow land (49 kg ha^{-1}), crimson clover (35 kg ha^{-1}) and field bean (30 kg ha^{-1}) compared to oat (24 kg ha^{-1}) and maize (25 kg ha^{-1}), whereas, the nitrate of the soil profile (0-60 cm) was not responded to the mineral NPK fertilization (p -value = 0.384) (table 9).

Table 9: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO_3^-) N of the soil in September 2018, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Nitrate (NO_3^-) N content of the soil (kg ha^{-1})		
		0-30 cm	30-60 cm	Sum 0-60 cm
Preceding crop (PC)	Fallow	39.1 a	9.9 a	49.0 a
	Crimson clover	28.6 b	6.7 b	35.3 b
	Field bean	23.6 b	6.1 bc	29.7 bc
	Oat	21.4 b	2.3 d	23.7 c
	Maize	21.8 b	3.4 cd	25.2 c
NPK fertilization (NPK)	No fertilization	24.6 ns	5.9 ns	30.5 ns
	PK 50%	27.2 ns	4.8 ns	32.0 ns
	PK+50% N	26.9 ns	5.3 ns	32.2 ns
	PK+100% N	28.8 ns	6.7 ns	35.5 ns
p -value	PC	< 0.001	< 0.001	< 0.001
	NPK	0.383	0.217	0.384
	PCxNPK	0.375	< 0.001	0.138
LSD 5% (in kg ha^{-1})	PC	7.7	2.9	6.7
	NPK	6.4	2.4	6.0
	PCxNPK	10.9	4.1	13.3

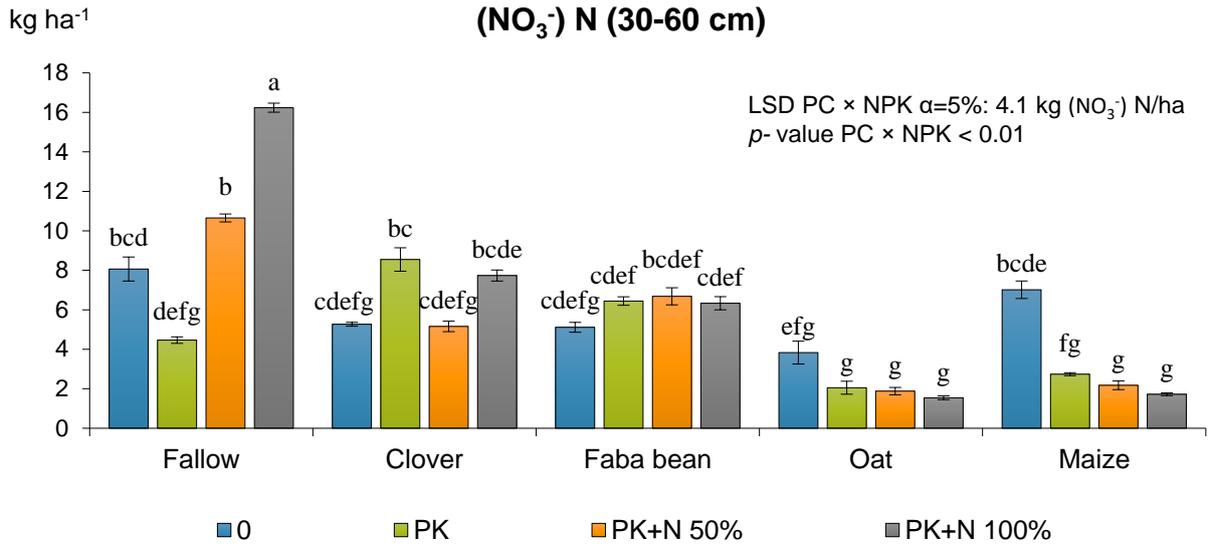


Figure 13: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO₃⁻) N of the subsoil (30-60 cm) (September 2018), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

November 2018

In November 2018, three weeks after incorporating the plant biomass of the pre-crops significant effects of preceding crops were found on (NO₃⁻) N in the topsoil (0-30 cm) (*p*-value < 0.001) (table 10). Crimson clover as a green mulch has increased the (NO₃⁻) N to the maximum level of 87 kg ha⁻¹. In contrary, after oat the lowest (29.4 kg ha⁻¹) level of (NO₃⁻) N was achieved. In addition, the (NO₃⁻) N of the topsoil was significantly affected by NPK fertilization (*p*-value = 0.044). PK+100% N (56 kg ha⁻¹), PK+50% N (52 kg ha⁻¹) and no fertilization (50 kg ha⁻¹) caused the same level of (NO₃⁻) N (table 10). So far, no significant effects on (NO₃⁻) N of the topsoil (0-30 cm) by the main factors (pre-crops and NPK fertilization) were found (*p*-value = 0.125).

The (NO₃⁻) N of the subsoil (30-60 cm) was only influenced by pre-crops. Particularly after fallow (20 kg ha⁻¹) higher (NO₃⁻) N level was achieved compared to the other treatment (table 10).

Looking at both layers (0-60 cm), it becomes clear that crimson clover used as a green mulch has accumulated the highest nitrate (NO₃⁻) N amount (103 kg ha⁻¹) in the soil profile, while field bean (57 kg ha⁻¹), oat (40 kg ha⁻¹) and maize (53 kg ha⁻¹) led to lower nitrate amounts compared to no fertilization (75 kg ha⁻¹). In addition, NPK fertilization was not influenced the nitrate level in the soil profile (0-60 cm) (*p*-value = 0.053).

Table 10: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO₃⁻) N of the soil in November 2018, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Nitrate (NO ₃ ⁻) N content of the soil (kg ha ⁻¹)		
		November 2018		
		0-30 cm	30-60 cm	Sum 0-60 cm
Preceding crop (PC)	Fallow	55.3 b	19.7 a	75.0 b
	Crimson clover	87.2 a	15.8 ab	103.0 a
	Field bean	43.6 c	13.2 bc	56.8 c
	Oat	29.4 d	10.3 c	39.7 d
	Maize	41.9 c	10.9 bc	52.8 c
NPK fertilization (NPK)	No fertilization	50.2 ab	12.6 ns	62.8 ns
	PK 50%	47.3 b	14.5 ns	61.8 ns
	PK+50% N	51.9 ab	13.3 ns	65.2 ns
	PK+100% N	56.5 a	15.6 ns	72.1 ns
<i>p</i> -value	PC	< 0.001	< 0.001	< 0.001
	NPK	0.044	0.285	0.053
	PCxNPK	0.125	0.968	0.244
LSD 5% (in kg ha ⁻¹)	PC	10.1	5.2	9.0
	NPK	8.5	4.4	8.0
	PCxNPK	14.4	7.5	18.0

March 2019

In March 2019, neither preceding crops (*p*-value = 0.569) nor NPK fertilization (*p*-value = 0.671) was significantly influenced the (NO₃⁻) N of the topsoil (0-30 cm) (table 11). The nitrate level varied from 11 to 24 kg ha⁻¹ in the topsoil (0-30 cm) among treatments (data not shown). So far, no significant interactions were found between the main factors (pre-crops and NPK fertilization) (*p*-value = 0.344).

In the second depth (30-60 cm) nitrate (NO₃⁻) N of the soil was significantly influenced by preceding crops (*p*-value < 0.001) (table 11). The highest (NO₃⁻) N was achieved by crimson clover used as a green mulch (20 kg ha⁻¹), while field bean (11 kg ha⁻¹), oat (7.5 kg ha⁻¹) and maize (9 kg ha⁻¹) obtained the same (NO₃⁻) N value as fallow (12 kg ha⁻¹). Furthermore, no significant effects of NPK fertilization were found on (NO₃⁻) N of the soil (*p*-value = 0.537).

In the third depth of the soil (60-90 cm), the same tendency was found like in the second depth (table 11). The (NO₃⁻) N of the soil was significantly influenced by preceding crops (*p*-value < 0.001). Crimson clover used as a green mulch achieved the highest (NO₃⁻) N level (41 kg ha⁻¹) whereas after field bean (21 kg ha⁻¹), maize (15 kg ha⁻¹) and fallow (18 kg ha⁻¹) lower (NO₃⁻) N values and after oat (9 kg ha⁻¹) the lowest (NO₃⁻) N amount was found. In addition, no significant effect was found by NPK fertilization (*p*-value = 0.229).

The analysis of variance has shown that in the first year after cultivating preceding crops crimson clover used as a green mulch significantly increased (80 kg ha⁻¹) the nitrate (NO₃⁻) N through the soil profile (0-90 cm) compared to field bean (46 kg ha⁻¹), fallow (44 kg ha⁻¹), maize

(40 kg ha⁻¹) and oat (34 kg ha⁻¹). So far, NPK fertilization was not influenced the nitrate level of the soil profile (0-90 cm) (p -value = 0.200).

Table 11: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO₃⁻) N of the soil (March 2019), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Nitrate (NO ₃ ⁻) N content of the soil (kg ha ⁻¹)			
		March 2019			
		0-30 cm	30-60 cm	60-90 cm	Sum 0-90 cm
Preceding crop (PC)	Fallow	14.6 ns	11.7 b	17.7 b	44.0 b
	Crimson clover	18.3 ns	20.5 a	40.8 a	79.6 a
	Field bean	14.3 ns	11.0 b	20.7 b	46.0 b
	Oat	17.3 ns	7.5 b	8.8 c	33.6 c
	Maize	16.8 ns	8.9 b	15.0 b	40.7 bc
NPK fertilization (NPK)	No fertilization	15.7 ns	11.9 ns	19.4 ns	47.0 ns
	PK 50%	15.2 ns	11.0 ns	20.3 ns	46.5 ns
	PK+50% N	18.1 ns	11.7 ns	19.0 ns	48.8 ns
	PK+100% N	15.9 ns	13.0 ns	23.7 ns	52.6 ns
p -value	PC	0.569	< 0.001	< 0.001	< 0.001
	NPK	0.671	0.537	0.229	0.501
	PC×NPK	0.344	0.339	0.139	0.200
LSD 5% (in kg ha ⁻¹)	PC	8.0	4.6	5.6	10.1
	NPK	6.7	3.8	6.6	9.0
	PC×NPK	11.4	6.5	11.2	20.2

October 2019

In October 2019, preceding crops were significantly influenced the (NO₃⁻) N of the topsoil (0-30 cm) indicated by p -value < 0.001. Crimson clover (29 kg ha⁻¹) and oat (32 kg ha⁻¹) achieved higher (NO₃⁻) N of the soil (0-30 cm) compared to field bean (27 kg ha⁻¹), maize (25 kg ha⁻¹) and fallow land (24 kg ha⁻¹) (table 12). Furthermore, significant effects of NPK fertilization were found on (NO₃⁻) N of the topsoil (0-30 cm) (p -value < 0.001). The optimal level of NPK fertilization (PK+100% N) led to the highest (NO₃⁻) N of the topsoil (34 kg ha⁻¹), while the split level of mineral N (PK+50% N) obtained the same value of (NO₃⁻) N (24 kg ha⁻¹) as PK 50% (27 kg ha⁻¹) and no fertilization (25 kg ha⁻¹). So far, no significant interactions were found between main factors (pre-crops and NPK fertilization) (p -value = 0.551). The (NO₃⁻) N level of the topsoil (0-30 cm) varied from 20 to 39 kg ha⁻¹ respectively (data not shown).

In the second depth of the soil (30-60 cm), pre-crops significantly affected the (NO₃⁻) N content of the soil (30-60 cm) indicated by p -value < 0.001 (table 12). Crimson clover (40 kg ha⁻¹), field bean (35 kg ha⁻¹) and oat (36 kg ha⁻¹) achieved the higher (NO₃⁻) N levels of the soil (30-60 cm) compared to maize (30 kg ha⁻¹) and fallow (30 kg ha⁻¹) (table 12). Furthermore, the optimal level of NPK fertilization (PK+100% N) obtained the higher (NO₃⁻) N of the soil (38 kg ha⁻¹) compared to no fertilization (36 kg ha⁻¹), PK 50% (31 kg ha⁻¹) and PK+50% N (31 kg ha⁻¹). So far, no significant interactions were found between the main factors (pre-crops and NPK

fertilization) (p -value = 0.178). The (NO_3^-) N level of the soil (30-60 cm) varied from 25 to 54 kg ha^{-1} (data not shown).

In the third depth of the soil (60-90 cm), significant effects on (NO_3^-) N of the subsoil (60-90 cm) by preceding crops (p -value = 0.0170) (table 12). Therefore, crimson clover (10 kg ha^{-1}) achieved the higher (NO_3^-) N values of the subsoil (60-90 cm) compared to field bean (8 kg ha^{-1}), maize (8 kg ha^{-1}), oat (7 kg ha^{-1}) and fallow (7 kg ha^{-1}). In addition, the (NO_3^-) N of the subsoil (60-90 cm) positively responded to mineral N (p -value < 0.001). The optimal level of NPK fertilization (PK+100% N) achieved the highest (NO_3^-) N level (11 kg ha^{-1}) of the subsoil (60-90 cm) compared to no fertilization (8 kg ha^{-1}). So far, significant interactions were found between the main factors (pre-crops and NPK fertilization). The (NO_3^-) N level of the subsoil (60-90 cm) varied from 5 to 18 kg ha^{-1} (figure 14).

The analysis of variance showed that in October 2019, three weeks after harvesting winter wheat, the nitrate content of the soil profile (0-90 cm) was increased by crimson clover (80 kg ha^{-1}), field bean (70 kg ha^{-1}) and oat (75 kg ha^{-1}) compared to maize (64 kg ha^{-1}) and fallow (61 kg ha^{-1}) (p -value < 0.001) (table 12). Furthermore, the optimal level of NPK fertilization (PK+100% N) achieved a significant higher (p -value < 0.001) level of nitrate (83 kg ha^{-1}) in the soil profile (0-90 cm).

Table 12: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO_3^-) N of the soil (October 2019), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Nitrate (NO_3^-) N content of the soil (kg ha^{-1})			
		October 2019			
		0-30 cm	30-60 cm	60-90 cm	Sum 0-90 cm
Preceding crop (PC)	Fallow	23.8 c	29.7 c	7.1 b	60.6 b
	Crimson clover	29.5 ab	39.8 a	10.3 a	79.6 a
	Field bean	27.4 bc	34.8 abc	8.0 b	70.2 ab
	Oat	31.9 a	35.9 ab	7.2 b	75.0 a
	Maize	25.4 c	29.9 bc	8.3 ab	63.6 b
NPK fertilization (NPK)	No fertilization	25.0 b	35.6 ab	8.3 b	68.9 b
	PK 50%	27.1 b	30.6 b	6.5 b	64.2 b
	PK+50% N	24.5 b	31.5 b	7.3 b	63.3 b
	PK+100% N	33.7 a	38.4 a	10.6 a	82.7 a
p -value	PC	< 0.001	< 0.001	0.0170	< 0.001
	NPK	< 0.001	0.025	< 0.001	< 0.001
	PCxNPK	0.551	0.178	< 0.001	0.100
LSD 5% (in kg ha^{-1})	PC	4.1	6.3	2.0	10.0
	NPK	3.7	5.6	1.8	9.0
	PCxNPK	8.2	12.6	4.0	20.0

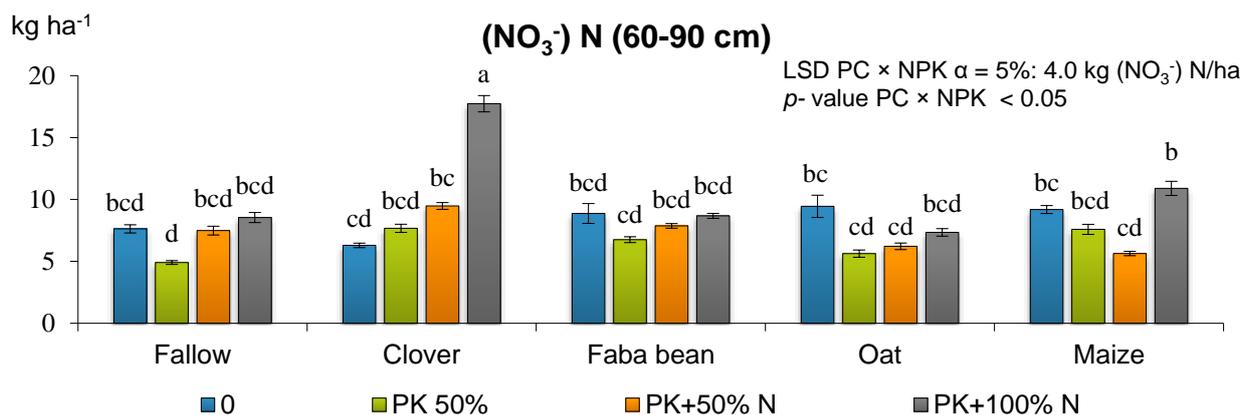


Figure 14: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO_3^-) N of the subsoil (60-90 cm) (October 2019), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

April 2020

In April 2020, no significant effects were found by preceding crops on (NO_3^-) N of the topsoil (0-30 cm) (p -value = 0.299). The (NO_3^-) N level of the topsoil recorded at 38 to 49 kg ha⁻¹ (table 13). In contrary, a positive relationship was found between mineral N fertilization and (NO_3^-) N of the topsoil (0-30 cm) (p -value < 0.001). It is found that both levels of mineral N, PK+100% N (63 kg ha⁻¹) and PK+50% N (47 kg ha⁻¹) achieved the higher levels of (NO_3^-) N of the soil (0-30 cm) compared to PK 50% (32 kg ha⁻¹) and no fertilization (36 kg ha⁻¹). So far, no significant interactions were found between the main factors (Pre-crops and NPK fertilization) (p -value = 0.879). The (NO_3^-) N level of the topsoil (0-30 cm) varied from 23 to 74 kg ha⁻¹ (data not shown).

In the second depth of the soil (30-60 cm), the same tendency was found comparing the treatments. Thus, no significant effects were observed by pre-crops on (NO_3^-) N of the soil (30-60 cm). The (NO_3^-) N level of the soil (30-60 cm) recorded at 18 to 22 kg ha⁻¹ by pre-crops. Furthermore, NPK fertilization significantly affected the (NO_3^-) N in the second layer of the soil (30-60 cm) (p -value < 0.001) (table 13). The optimal level of NPK fertilization (PK+100% N) increased the (NO_3^-) N of the soil to the maximum level of 25 kg ha⁻¹. Afterwards, PK+50% N (16 kg ha⁻¹), PK 50% (20 kg ha⁻¹) and no fertilization (18 kg ha⁻¹) achieved the same (NO_3^-) N value of the soil (30-60 cm) (table 13). So far, no significant interactions were found between the main factors (pre-crops and NPK fertilization) on (NO_3^-) N of the soil (30-60 cm) (p -value = 0.899). The (NO_3^-) N level of the soil (30-60 cm) varied from 12 to 27 kg ha⁻¹ (data not shown).

In the third depth of the soil (60-90 cm), preceding crops significantly increased the (NO_3^-) N of the subsoil (p -value < 0.001) (table 13). Therefore, crimson clover used as a green mulch significantly increased the (NO_3^-) N of the subsoil (60-90 cm) to the maximum level of (18 kg ha⁻¹). Afterwards, oat (14 kg ha⁻¹) obtained the higher (NO_3^-) N of the subsoil compared to field bean (11 kg ha⁻¹), maize (11 kg ha⁻¹) and fallow (11 kg ha⁻¹) (table 13). In addition, the (NO_3^-) N of the subsoil (60-90 cm) significantly affected by NPK fertilization (p -value < 0.001). The optimal level of NPK fertilization (PK+100% N) achieved the higher (NO_3^-) N of the subsoil at the level of (18 kg ha⁻¹) compared to PK+50% N (12 kg ha⁻¹), PK 50% (10 kg ha⁻¹) and no fertilization (11 kg ha⁻¹) (table 13). So far, significant interactions were found between the main

factors (pre-crops and NPK fertilization) (p -value < 0.001). The (NO_3^-) N level of the subsoil (60-90 cm) varied from 6 to 31 kg ha^{-1} (figure 15).

The nitrate content of the whole soil profile (0-90 cm) was not influenced by preceding crops in April 2020, the second year after the cultivation of pre-crops (p -value = 0.057), while the nitrate of the soil profile showed a positive response to mineral NPK fertilization (p -value < 0.001), and the optimal level of NPK fertilization (PK+100% N) achieved the highest nitrate value (107 kg ha^{-1}) in the soil compared to no fertilization (65 kg ha^{-1}). In addition, no significant effects were found between the main factors (pre-crops and NPK fertilization) (p -value = 0.601).

Table 13: Effect of preceding crops (PC) and NPK fertilization (NPK) on nitrate (NO_3^-) N of the soil in April 2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Nitrate (NO_3^-) N content of the soil (kg ha^{-1})			
		April 2020			
		0-30 cm	30-60 cm	60-90 cm	Sum 0-90 cm
Preceding crop (PC)	Fallow	48.5 ns	18.3 ns	11.0 c	77.8 ns
	Crimson clover	49.5 ns	20.3 ns	18.3 a	88.1 ns
	Field bean	38.5 ns	18.4 ns	11.1 c	68.0 ns
	Oat	44.6 ns	22.6 ns	14.0 b	81.2 ns
	Maize	42.7 ns	19.3 ns	10.6 c	72.6 ns
NPK fertilization (NPK)	No fertilization	35.9 c	17.9 b	11.2 bc	65.0 bc
	PK 50%	32.4 c	19.9 b	9.8 c	62.1 c
	PK+50% N	47.5 b	16.1 b	12.5 b	76.1 b
	PK+100% N	63.2 a	25.3 a	18.4 a	106.9 a
p -value	PC	0.299	0.519	< 0.001	0.057
	NPK	< 0.001	< 0.001	< 0.001	< 0.001
	PC×NPK	0.879	0.899	< 0.001	0.601
LSD 5% (in kg ha^{-1})	PC	11.4	5.5	2.0	14.1
	NPK	10.2	4.9	1.8	12.6
	PC×NPK	22.7	11.0	3.9	28.1

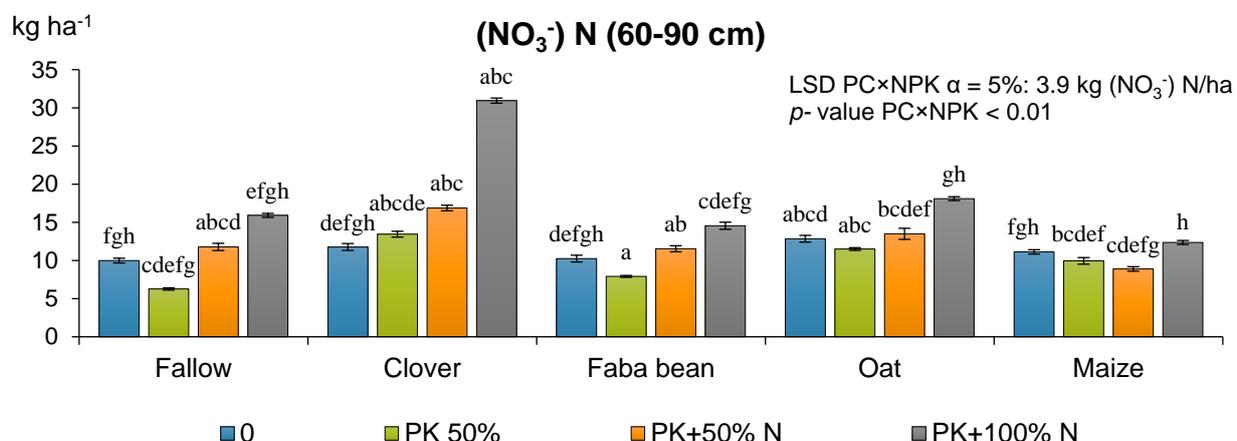


Figure 15: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on nitrate (NO_3^-) N of the subsoil (60-90 cm) (April 2019), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

4. 1. 2 Total nitrogen (Nt) concentration of the soil

The first Nt analysis was carried out with soil samples taken from 0-20 and 20-40 cm in **September 2015** (table 14). The Nt of the topsoil (0-20 cm) varied from 0.141 to 0.174% w/w (data not shown). It was found that in the upper layer (0-20 cm) Nt was significantly affected by preceding crops (p -value < 0.001) caused by crimson clover as a green mulch that has increased the Nt of the soil to the maximum level of 0.165% w/w. After field bean (0.161% w/w) and maize (0.159% w/w) the same value as crimson clover was achieved, while after oat (0.155% w/w) and fallow (0.155% w/w) the lowest level of Nt in the topsoil was observed.

In addition, significant effects on Nt were found by mineral fertilization (NPK) (p -value < 0.001) (table 14). Both nitrogen fertilised treatments (PK+50% N and PK+100% N) achieved higher Nt level of the topsoil (0.169 and 0.160% w/w respectively) compared to PK 50% (0.151% w/w) and no fertilization (0.157% w/w).

In the second depth (20-40 cm), preceding crops were significantly increased the Nt concentration of the subsoil (20-40 cm) (p -value < 0.001) (table 14). Crimson clover used as a green mulch (0.169% w/w), field bean (0.164% w/w), oat (0.161% w/w) and maize (0.159% w/w) achieved higher Nt level compared to fallow (0.152% w/w). Furthermore, PK+50% N (0.166% w/w), PK+100% N (0.164% w/w) and no fertilization (0.161% w/w) obtained highest Nt values in 20-40 cm compared to PK 50% (0.152% w/w).

Table 14: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt concentration of the soil (September 2015), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment, ns: not significant.

Treatments		Total nitrogen (Nt) concentration of the soil (% w/w)	
		September 2015	
		0-20 cm	20-40 cm
Preceding crop (PC)	Fallow	0.155 b	0.152 b
	Crimson clover	0.165 a	0.169 a
	Field bean	0.161 ab	0.164 a
	Oat	0.155 b	0.161 ab
	Maize	0.159 ab	0.159 ab
NPK fertilization (NPK)	No fertilization	0.157 b	0.161 ab
	PK 50%	0.151 b	0.152 b
	PK+50% N	0.169 a	0.166 a
	PK+100% N	0.160 ab	0.164 a
p -value	PC	< 0.001	< 0.001
	NPK	< 0.001	< 0.001
	PCxNPK	0.460	0.513
LSD 5% (in % w/w)	PC	0.007	0.009
	NPK	0.006	0.008
	PCxNPK	0.010	0.013

In **September 2016** the Nt concentration of the soil (0-30 cm) varied only slightly from 0.168 to 0.179% w/w (table 15). Neither preceding crops (p -value = 0.424) nor NPK fertilization (p -value = 0.106) was significantly affected Nt concentration of the topsoil at this time. Furthermore, no significant effects were found between the main factors (pre-crops and NPK fertilization) (p -value = 0.843).

Table 15: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt level of the soil (0-30 cm) (September 2016), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment, ns: not significant.

Total nitrogen (Nt) concentration of the soil (% w/w) - September 2016							
	Fallow	Clover	Field bean	Oat	Maize	p -value	LSD 5% (in % w/w)
No fertilization	0.170 ns	0.173 ns	0.177 ns	0.168 ns	0.173 ns	0.42	0.007
PK+50% N	0.168 ns	0.179 ns	0.175 ns	0.176 ns	0.178 ns	0.11	0.005
PK+100% N	0.176 ns	0.177 ns	0.178 ns	0.179 ns	0.179 ns	0.84	0.012

The third Nt analysis of soil samples taken in **September 2018** (0-30 cm) has expressed a variation from 0.152 to 0.170% w/w among the treatments (data not shown). The statistical analysis has shown that crimson clover (0.165% w/w), field bean (0.163% w/w), oat (0.161% w/w) and maize (0.161% w/w) significantly increased (p -value < 0.05) the Nt of the topsoil compared to fallow (0.154% w/w) (table 16). Furthermore, significant effects on total N were also found by mineral NPK fertilization (p -value < 0.05) indicating an increase to 0.164% w/w (PK+50% N). In the soil depth 30-60 cm Nt varied from 0.109 to 0.133% w/w among the treatments. Neither pre-crops (p -value = 0.645) nor NPK fertilization (p -value = 0.343) has shown clear effects on total N of this soil (30-60 cm).

Table 16: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt level of the soil (September 2018), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment, ns: not significant.

Treatments		Total nitrogen (Nt) concentration of the soil (% w/w)	
		September 2018	
		0-30 cm	30-60 cm
Preceding crop (PC)	Fallow	0.154 b	0.118 ns
	Crimson clover	0.165 a	0.119 ns
	Field bean	0.163 ab	0.120 ns
	Oat	0.161 ab	0.118 ns
	Maize	0.161 ab	0.125 ns
NPK fertilization (NPK)	No fertilization	0.161 ab	0.116 ns
	PK 50%	0.156 b	0.120 ns
	PK+50% N	0.164 a	0.118 ns
	PK+100% N	0.162 a	0.125 ns
p -value	PC	0.011	0.645
	NPK	0.018	0.343
	PCxNPK	0.919	0.870
LSD 5% (in % w/w)	PC	0.008	0.016
	NPK	0.007	0.013
	PCxNPK	0.011	0.022

In **November 2018**, three weeks after incorporating the plant biomass into the soil the Nt values of the topsoil (0-30 cm) varied from 0.153 to 0.177% w/w among the factors pre-crops and NPK fertilization (data not shown). Based on the result (table 17) significant effects of preceding crops were found (p -value < 0.001) indicating increased Nt concentrations after crimson clover (0.172% w/w), field bean (0.165% w/w), oat (0.163% w/w) and maize (0.163% w/w) compared to fallow (0.158% w/w). Furthermore, both levels of mineral N, PK+50% N (0.168% w/w) and PK+100% N (0.168% w/w) were also significantly increased the Nt of the topsoil (p -value < 0.001) compared to PK 50% (0.159% w/w) and n (0.161% w/w each).

In the subsoil (30-60 cm), neither by preceding crops (p -value = 0.458) nor by mineral NPK fertilization (p -value = 0.181) the Nt of the subsoil was affected. Furthermore, no significant interactions were found by the main factors (pre-crops and mineral NPK fertilization) (p -value = 0.727). The Nt value of the subsoil (30-60 cm) varied from 0.113 to 0.140% w/w among treatments.

Table 17: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt concentration of the soil in November 2018, LTE “BNF” Giessen. Different letters indicate significant differences among the means treatment, ns: not significant.

Treatments		Total nitrogen (Nt) concentration of the soil (% w/w)	
		November 2018	
		0-30 cm	30-60 cm
Preceding crop (PC)	Fallow	0.158 b	0.124 ns
	Crimson clover	0.172 a	0.130 ns
	Field bean	0.165 ab	0.123 ns
	Oat	0.163 ab	0.122 ns
	Maize	0.163 ab	0.128 ns
NPK fertilization (NPK)	No fertilization	0.161 b	0.130 ns
	PK 50%	0.159 b	0.121 ns
	PK+50% N	0.168 a	0.126 ns
	PK+100% N	0.168 a	0.123 ns
p -value	PC	< 0.001	0.458
	NPK	< 0.001	0.181
	PCxNPK	0.551	0.727
LSD 5% (in % w/w)	PC	0.006	0.010
	NPK	0.005	0.009
	PCxNPK	0.009	0.019

In **March 2019**, the first year after preceding crops, the Nt level of the topsoil (0-30 cm) varied from 0.155 to 0.180% w/w (data not shown). Based on the results (table 18), crimson clover (0.173% w/w), field bean (0.168% w/w), oat (0.169% w/w) and maize (0.167% w/w) significantly increased Nt concentration in the topsoil (0-30 cm) compared to fallow (0.160% w/w) (p -value < 0.001). In addition, mineral N, represented by the treatments PK+50% N (0.171% w/w) and PK+100% N (0.171% w/w) has significantly increased the total N of the topsoil compared to no fertilization (0.163% w/w).

In the second soil depth (30-60 cm) the total N concentration varied from 0.115 to 0.150% w/w among the treatments (data not shown). Only preceding crops were influenced the total N

of the soil (p -value < 0.001) (table 18). Crimson clover (0.139% w/w), field bean (0.131% w/w), oat (0.126% w/w) and maize (0.134% w/w) achieved higher Nt of the topsoil compared to fallow (0.119% w/w) (table 18). In the deepest soil layer (60-90 cm), neither preceding crops (p -value = 0.559) nor NPK fertilization (p -value = 0.282) affected the total N. Furthermore, no significant interactions were found between the main factors (pre-crops and NPK fertilization) (p -value = 0.808).

Table 18: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt level of the soil in March 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment, ns: not significant.

Treatments		Total nitrogen (Nt) concentration of the soil (% w/w)		
		March 2019		
		0-30 cm	30-60 cm	60-90 cm
Preceding crop (PC)	Fallow	0.160 c	0.119 b	0.103 ns
	Crimson clover	0.173 a	0.139 a	0.102 ns
	Field bean	0.168 b	0.131 ab	0.098 ns
	Oat	0.169 ab	0.126 ab	0.096 ns
	Maize	0.167 b	0.134 ab	0.103 ns
NPK fertilization (NPK)	No fertilization	0.163 b	0.135 ns	0.104 ns
	PK 50%	0.164 b	0.126 ns	0.095 ns
	PK+50% N	0.171 a	0.131 ns	0.099 ns
	PK+100% N	0.171 a	0.127 ns	0.103 ns
p -value	PC	< 0.001	< 0.001	0.559
	NPK	< 0.001	0.246	0.282
	PCxNPK	0.306	0.924	0.808
LSD 5% (in % w/w)	PC	0.005	0.011	0.011
	NPK	0.004	0.010	0.010
	PCxNPK	0.009	0.022	0.021

In **October 2019**, the Nt level of the upper soil (0-30 cm) varied from 0.163 to 0.185% w/w (data not shown). Based on the analysed results (table 19), a significant effect by preceding crops on Nt in topsoil (0-30 cm) was found (p -value < 0.001). Crimson clover (0.178% w/w) and field bean (0.174% w/w) increased the Nt compared to oat (0.173% w/w), maize (0.169% w/w) and fallow (0.167% w/w). Furthermore, the total N concentration of the topsoil positively responded to mineral NPK fertilization (p -value < 0.001). PK+50% N (0.176% w/w) and PK+100% N (0.176% w/w) achieved the higher concentration of total N in the topsoil compared to no fertilization (0.169% w/w) (table 19).

In contrary, in the second depth (30-60 cm), the Nt concentration was influenced only by NPK fertilization (p -value < 0.05) but with an ambiguous trend. PK+50% N (0.142% w/w) and no fertilization (0.143% w/w) obtained the higher total N concentration compared to PK+100% N (0.137% w/w) and PK 50% (0.134% w/w) (table 19).

In the third depth (60-90 cm), neither preceding crops (p -value = 0.611) nor NPK fertilization (p -value = 0.200) affected the Nt concentration in the deepest soil layer.

Table 19: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt concentration of the soil (October 2019), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment, ns: not significant.

Treatments		Total nitrogen (Nt) concentration of the soil (% w/w)		
		October 2019		
		0-30 cm	30-60 cm	60-90 cm
Preceding crop (PC)	Fallow	0.167 d	0.137 ns	0.097 ns
	Crimson clover	0.178 a	0.143 ns	0.097 ns
	Field bean	0.174 ab	0.139 ns	0.096 ns
	Oat	0.173 bc	0.138 ns	0.093 ns
	Maize	0.169 cd	0.140 ns	0.104 ns
NPK fertilization (NPK)	No fertilization	0.169 b	0.143 a	0.098 ns
	PK 50%	0.166 b	0.134 c	0.093 ns
	PK+50% N	0.176 a	0.142 ab	0.093 ns
	PK+100% N	0.176 a	0.137 bc	0.105 ns
<i>p</i> -value	PC	< 0.001	0.443	0.611
	NPK	< 0.001	0.025	0.200
	PC×NPK	0.669	0.951	0.861
LSD 5% (in % w/w)	PC	0.005	0.007	0.014
	NPK	0.004	0.006	0.012
	PC×NPK	0.009	0.014	0.027

In **April 2020**, the Nt level of the topsoil (0-30 cm) varied from 0.165 to 0.192% w/w (considering all plots, data not shown). Based on the analysed data (table 20), the total N concentration of the topsoil (0-30 cm) was significantly affected by both preceding crops (*p*-value < 0.001) and NPK fertilization (*p*-value < 0.001). Hence, crimson clover (0.184% w/w), field bean (0.177% w/w), oat (0.176% w/w) and maize (0.179% w/w) significantly increased the Nt concentration compared to fallow (0.170% w/w). On the other hand, PK+100% N (0.182% w/w) and PK+50% N (0.181% w/w) achieved higher levels of total N concentration compared to PK 50% (0.171% w/w) and no fertilization (0.173% w/w). No significant effects were found between the main factors (pre-crops and NPK fertilization) (*p*-value = 0.174).

In the second depth (30-60 cm) Nt concentration varied from 0.127 to 0.150% w/w between the treatments (data not shown) that had no influence on this soil parameter (table 20). The values in 60-90 cm, which were also not influenced by the variants, were even lower 0.099-0.110% w/w.

Table 20: Effect of preceding crops (PC) and NPK fertilization (NPK) on Nt concentration of the soil in April 2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment, ns: not significant.

Treatments		Total nitrogen (Nt) concentration of the soil (% w/w)		
		April 2020		
		0-30 cm	30-60 cm	60-90 cm
Preceding crop (PC)	Fallow	0.170 c	0.133 ns	0.099 ns
	Crimson clover	0.184 a	0.139 ns	0.104 ns
	Field bean	0.177 b	0.137 ns	0.099 ns
	Oat	0.176 b	0.138 ns	0.099 ns
	Maize	0.179 b	0.144 ns	0.106 ns
NPK fertilization (NPK)	No fertilization	0.173 b	0.138 ns	0.103 ns
	PK 50%	0.171 b	0.135 ns	0.099 ns
	PK+50% N	0.181 a	0.140 ns	0.101 ns
	PK+100% N	0.182 a	0.139 ns	0.103 ns
<i>p</i> -value	PC	< 0.001	0.130	0.494
	NPK	< 0.001	0.672	0.808
	PC×NPK	0.174	0.930	0.596
LSD 5% (in % w/w)	PC	0.004	0.009	0.010
	NPK	0.004	0.008	0.009
	PC×NPK	0.009	0.017	0.021

4. 1. 3 Dissolved organic nitrogen (DON)

The first DON measurement was carried out with soil samples taken in **September 2018**, two weeks after harvesting the last preceding crop. The DON level of the topsoil varied from 10.3 to 31.9 kg ha⁻¹ (considering the individual plots, data not shown). Based on the analysed data (table 21), DON of the topsoil (0-30 cm) was not significantly affected by pre-crops (*p*-value = 0.674) but significant differences have been found by different mineral fertilizations (*p*-value = 0.016). Despite observing no significant effect by pre-crops, the DON value of clover mulch (19.4 kg ha⁻¹) and field bean (19.1 kg ha⁻¹) was slightly higher compared to oat and maize (18.8 kg ha⁻¹) or fallow land (17.3 kg ha⁻¹). On the other hand, PK+50% N (20.5 kg ha⁻¹), PK+100% N (19.3 kg ha⁻¹) significantly increased the DON of the soil (0-30 cm) compared to PK 50% (16.2 kg ha⁻¹) or no fertilization (18.3 kg ha⁻¹). No significant interaction effects were found between the main factors (pre-crop and NPK fertilization) (*p*-value = 0.369).

In the second layer of the soil (30-60 cm), the DON level varied from 9.2 to 30.4 kg ha⁻¹ (data shown). No significant effects were found by either pre-crops (*p*-value = 0.081) or mineral NPK fertilization (*p*-value = 0.181) (table 21). Furthermore, no significant interaction effects were found between the main factors (pre-crops and NPK fertilization) (*p*-value = 0.901).

Table 21: Effect of preceding crops (PC) and NPK fertilization (NPK) on DON of the soil in September 2018, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Dissolved organic nitrogen (DON) of the soil (kg ha ⁻¹)	
		September 2018	
		0-30 cm	30-60 cm
Preceding crop (PC)	Fallow	17.3 ns	13.2 ns
	Crimson clover	19.4 ns	14.9 ns
	Field bean	19.1 ns	16.3 ns
	Oat	18.5 ns	13.6 ns
	Maize	18.5 ns	15.9 ns
NPK fertilization (NPK)	No fertilization	18.3 ab	15.5 ns
	PK 50%	16.2 b	13.8 ns
	PK+50% N	20.5 a	15.9 ns
	PK+100% N	19.3 a	13.9 ns
<i>p</i> -value	PC	0.674	0.081
	NPK	0.016	0.181
	PC×NPK	0.369	0.901
LSD 5% (in kg ha ⁻¹)	PC	2.9	2.6
	NPK	2.6	2.3
	PC×NPK	5.9	5.2

The second DON analysis were carried out in **March 2019**, after incorporation of pre-crops residue in the soil and in early growing stage of winter wheat. Based on the analysed data, significant effects were found by different pre-crops (*p*-value = 0.034) (figure 16). Hence, crimson clover significantly increased the DON value of the topsoil to the maximum level of 18.4 kg ha⁻¹. The same identical but lower values have been observed by field bean (16.6 kg ha⁻¹) and oat (16.6 kg ha⁻¹), respectively. Maize (16.3 kg ha⁻¹) and fallow land (15.0 kg ha⁻¹) achieved the lowest DON of the topsoil. The DON level of the topsoil (0-30 cm) varied from 3.5 kg ha⁻¹ to 25.7 kg ha⁻¹ (considering the individual plots, data not shown).

In addition, no significant differences were found between different mineral NPK fertilization on DON values of the topsoil (*p*-value = 0.160). Based on the analysed data, no significant interaction effects were found between the main factors (pre-crops and NPK fertilization) (*p*-value = 0.542).

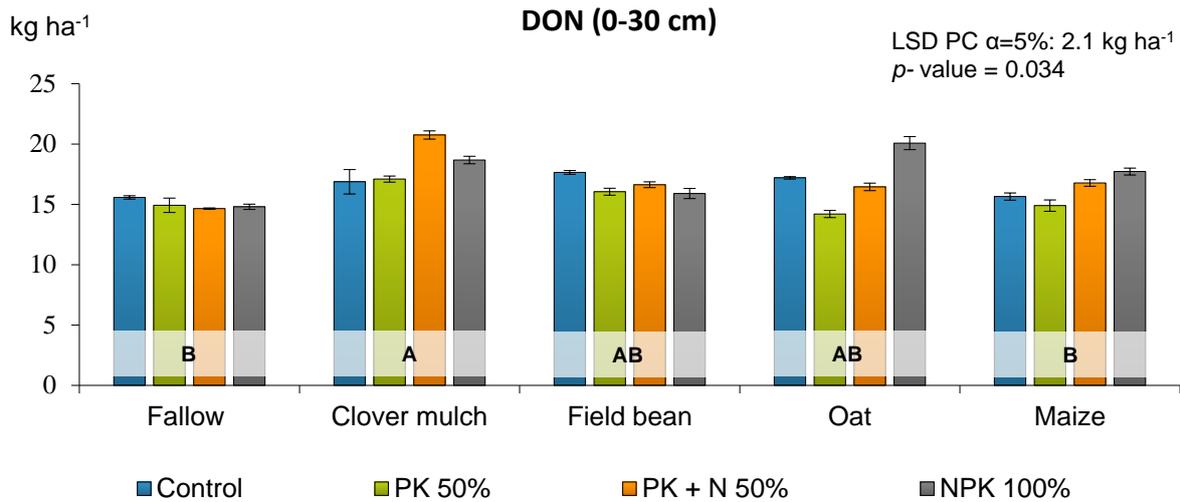


Figure 16: Effect of pre-crops (PC) on DON of the topsoil (0-30 cm) March 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

Table 22: Effect of preceding crops (PC) and NPK fertilization (NPK) on DON of the topsoil (0-30 cm) March 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

Dissolved organic nitrogen (DON) of the soil (kg ha ⁻¹) - March 2019 (0-30 cm)					
Treatments	No fertilization	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	15.6	14.9	14.7	14.8	15.0 b
Crimson clover	16.9	17.1	20.8	18.7	18.4 a
Field bean	17.7	16.1	16.6	15.9	16.6 ab
Oat	17.2	14.2	16.5	20.1	17.0 ab
Maize	15.7	14.9	16.8	17.7	16.3 b
Mean	16.6 ns	15.4 ns	17.1 ns	17.4 ns	
p-value	PC: 0.034		NPK: 0.160		PC x NPK: 0.542
LSD 5% (in kg ha ⁻¹)	PC: 2.1		NPK: 1.8		PC x NPK: 4.1

4. 1. 4 Concentration of Total Nitrogen bound (TNb) in the soil

In the current study the first TNb analysis of the topsoil (0-30 cm) was carried out in **November 2018**, three weeks after incorporating the plant biomass of the pre-crops into the soil. It was found that the TNb values varied from 2.6 to 5.4 mg/l (table 23). The statistical analysis has shown significant effects on TNb within this soil layer which were caused by preceding crops (p -value < 0.001) as well as by mineral NPK fertilization (p -value < 0.001). After crimson clover (4.7 mg/l) significantly higher TNb concentration was observed compared to field bean (3.2 mg/l), maize (3.1 mg/l), fallow (3.0 mg/l) and oat (2.9 mg/l). Furthermore, PK+50% N (3.6 mg/l), PK+100% N (3.5 mg/l) and fallow (3.5 mg/l) increased the TNb of the topsoil compared to PK 50% (2.9 mg/l).

Table 23: Effect of preceding crops (PC) and NPK fertilization (NPK) on TNb of the topsoil (0-30 cm) November 2018, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Total nitrogen bound (TNb) of the topsoil (0-30 cm) in mg/l					
Treatment	No fertilization	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	3.2 ns	2.6 ns	3.2 ns	3.2 ns	3.0 b
Crimson clover	4.3 ns	4.3 ns	5.4 ns	4.8 ns	4.7 a
Field bean	3.5 ns	2.5 ns	3.4 ns	3.5 ns	3.2 b
Oat	3.1 ns	2.4 ns	2.8 ns	3.3 ns	2.9 b
Maize	3.3 ns	2.9 ns	3.5 ns	2.6 ns	3.1 b
Mean	3.5 a	2.9 b	3.6 a	3.5 a	3.4
<i>p</i> -value	PC: < 0.001		NPK: < 0.001		PC x NPK: 0.054
LSD 5%	PC: 0.4 mg/l		NPK: 0.3 mg/l		PC x NPK: 0.7 mg/l

The second TNb analysis was carried out in **March 2019** at the time of juvenile growth of winter wheat plants. The TNb value of the topsoil (0-30 cm) varied from 1.4 to 2.4 mg/l between the treatments (*p*-value = 0.028) (figure 17). Based on the analysed data that are shown in table 24 the TNb of the topsoil was significantly affected by preceding crops (*p*-value < 0.001) and by mineral NPK fertilization (*p*-value < 0.001). In the treatments of crimson clover (2.0 mg/l), oat (1.8 mg/l) and maize (1.8 mg/l) an increased TNb of the topsoil was found compared to field bean (1.6 mg/l) and fallow (1.5 mg/l). In addition, the highest dosage of NPK fertilization (PK+100% N) has increased the TNb concentration of the topsoil to the maximum concentration of 2.0 mg/l, while in the treatments of PK+50% N (1.7 mg/l), PK 50% (1.7 mg/l) and no fertilization (1.6 mg/l) lower values were observed.

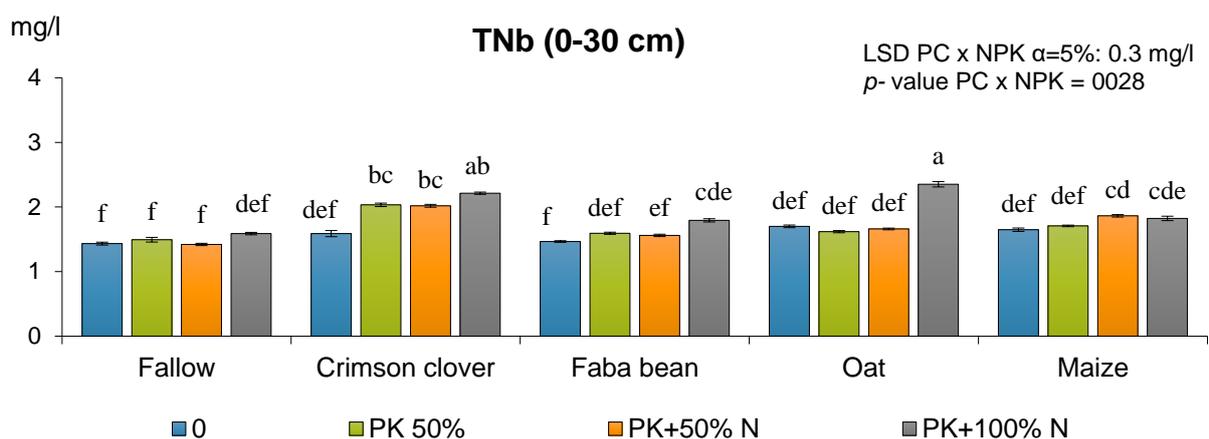


Figure 17: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on TNb of the topsoil (0-30 cm) in March 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

In the second soil depth (30-60 cm) the TNb varied between the treatments from 1.2 to 2.3 mg/l (data not shown). Based on statistical analysis only preceding crops had an effect on the TNb of the soil layer (*p*-value < 0.001) but not mineral NPK fertilization (*p*-value = 0.878). Therefore, the previous mulching with crimson clover (2.1 mg/l) has significantly increased the

TNb concentration compared to field bean (1.5 mg/l), oat (1.3 mg/l), maize (1.5 mg/l) and fallow (1.4 mg/l). No significant interaction effects were found between the main factors pre-crops and NPK fertilization (p -value = 0.403). In the third depth (60-90 cm) the TNb value of the subsoil varied from 1.1 to 2.8 mg/l between the treatments (data not shown). The analysis of variance revealed a significant effect on TNb of the soil caused by preceding crops (p -value < 0.001) as well as mineral NPK fertilization (p -value = 0.024) (table 24).

Again, crimson clover as mulching one year before (2.5 mg/l) has significantly increased the TNb of the soil compared to field bean (1.6 mg/l), Oat (1.2 mg/l), maize (1.4 mg/l) and fallow land (1.4 mg/l). In addition, PK+100% N (1.7 mg/l) and no fertilization (1.9 mg/l) led to increased TNb concentration of this soil layer compared to PK+50% N (1.4 mg/l) and PK 50% (1.4 mg/l). However, no significant interaction effects were found between the main factors (pre-crops and NPK fertilization) (p -value = 0.964).

Table 24: Effect of preceding crops (PC) and NPK fertilization (NPK) on TNb concentration of the soil in March 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Total nitrogen bound (TNb) of the soil (mg/l)		
		March 2019		
		0-30 cm	30-60 cm	60-90 cm
Preceding crop (PC)	Fallow	1.5 c	1.4 b	1.4 bc
	Crimson clover	2.0 a	2.1 a	2.5 a
	Field bean	1.6 c	1.5 b	1.6 b
	Oat	1.8 ab	1.3 b	1.2 c
	Maize	1.8 b	1.5 b	1.4 bc
NPK fertilization (NPK)	No fertilization	1.6 c	1.5 ns	1.9 a
	PK 50%	1.7 bc	1.6 ns	1.4 b
	PK+50% N	1.7 b	1.6 ns	1.4 b
	PK+100% N	2.0 a	1.6 ns	1.7 ab
p -value	PC	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	0.878	0.024
	PCxNPK	0.028	0.403	0.964
LSD 5%	PC	0.1 mg/l	0.2 mg/l	0.4 mg/l
	NPK	0.1 mg/l	0.2 mg/l	0.3 mg/l
	PCxNPK	0.3 mg/l	0.4 mg/l	0.8 mg/l

4. 1. 5 C/N ratio of the soil

It was found that the C/N ratio of the soil ranged from 8.7 to 10.2 (table 25). Within the 9th crop rotation, there was no significant effect on C/N ratio caused by preceding crops. However, in 2015 a positive effect of mineral fertilization (PK+100% N \geq PK 50%) was observed. In contrary to this, in the first year of the 10th crop rotation (in 9/2018) mineral fertilization led to higher C/N ratios varying from 10.0 to 10.2 compared to the control (not fertilized), which reached 9.3 (table 25). In addition, in 10/2019 and 4/2020 the previous crop cultivation caused modifications of the C/N ratio indicated by significant lower C/N ratio after wheat compared to fallow land and some other pre crops.

Table 25: C/N ratio of the topsoil depending on pre crops and NPK fertilization over the time from Sept 2015 to April 2020, LTE “BNF” Giessen.

Treatment	C/N ratio of the soil from Sept. 2015 to April 2020						
	9 th Crop rotation		10 th Crop rotation				
	9/2015	9/2016	9/2018	11/2018	3/2019	10/2019	4/2020
Fallow	9.5 ns	9.6 ns	10.1 ns	9.8 ns	9.4 ns	9.5 a	9.3 a
Clover mulch	9.2 ns	9.4 ns	9.7 ns	9.7 ns	9.3 ns	9.2 ab	9.1 ab
Field bean	9.4 ns	9.5 ns	9.9 ns	9.9 ns	9.5 ns	9.4 a	9.0 abc
Oat	9.3 ns	9.6 ns	10.0 ns	9.6 ns	9.4 ns	9.2 ab	8.8 bc
Maize	9.2 ns	9.2 ns	9.8 ns	9.6 ns	9.2 ns	9.0 b	8.7 c
<i>p</i> -value	0.564	0.735	0.503	0.679	0.586	0.020	0.008
No fertilization	9.2 b	9.3 ns	9.3 b	9.7 ns	9.4 ns	9.4 ns	9.0 ns
PK 50%	9.4 ab	-	10.0 ab	9.7 ns	9.3 ns	9.2 ns	8.9 ns
PK +50% N	9.1 b	9.7 ns	10.0 ab	9.7 ns	9.5 ns	9.3 ns	9.1 ns
PK+100% N	9.6 a	9.4 ns	10.2 a	9.8 ns	9.2 ns	9.1 ns	8.9 ns
<i>p</i> -value	0.025	0.443	0.001	0.917	0.410	0.111	0.323

4. 1. 6 Total Carbon (Ct) concentration of the soil

The Ct concentration of the soil was measured a total of seven times during the investigation period. Twice within the 9th crop rotation (in 2015 and 2016), twice directly after the previous crops (in September and November 2018) and three times in the 10th crop rotation in 2019 to 2020. The reason for these multiple measurements was to get representative results that enable the most reliable information possible about the Ct concentration in the soil. The results for the individual years are explained below.

The Ct concentration of the topsoil (0-20 cm) which was analysed in September 2015 varied from minimum 1.391 to maximum 1.629% w/w between the treatments (data not shown). The data have shown that the Ct was influenced only by NPK fertilization (*p*-value < 0.001) but not by preceding crop (*p*-value = 0.144) nor by the interaction between both factors (*p*-value = 0.680) (table 26). It was observed that PK+50% N (1.535% w/w) and PK+100% N (1.538% w/w) increased the Ct of the topsoil compared to PK 50% (1.421% w/w) and no fertilization (1.434% w/w).

In the second depth (20-40 cm) the same Ct level compared to 0-20 cm was found. Neither preceding crops (*p*-value = 0.236) nor NPK fertilization (*p*-value = 0.086) or the interaction between both factors (*p*-value 0.764) have influenced Ct concentration of this soil layer (table 26).

Table 26: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct concentration of the soil in September 2015, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Total carbon (Ct) concentration of the soil (% w/w)	
		0-20 cm	20-40 cm
Preceding crop (PC)	Fallow	1.471 ns	1.436 ns
	Crimson clover	1.523 ns	1.521 ns
	Field bean	1.517 ns	1.509 ns
	Oat	1.442 ns	1.462 ns
	Maize	1.457 ns	1.448 ns
NPK fertilization (NPK)	No fertilization	1.434 b	1.454 ns
	PK 50%	1.421 b	1.428 ns
	PK+50% N	1.535 a	1.526 ns
	PK+100% N	1.538 a	1.493 ns
<i>p</i> -value	PC	0.144	0.236
	NPK	< 0.001	0.086
	PC×NPK	0.680	0.764
LSD 5% (in % w/w)	PC	0.109	0.126
	NPK	0.091	0.105
	PC×NPK	0.155	0.178

The second Ct analysis was carried out with soil samples taken in **September 2016**, three weeks after harvesting winter rye. It was found that Ct concentration in 0-30 cm varied from 1.586 to 1.731% w/w (table 27). Neither preceding crops (*p*-value = 0.895) nor NPK fertilization (*p*-value = 0.148) or the interaction (*p*-value = 0.774) between both factors have affected the Ct of the soil.

Table 27: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct concentration of the soil (0-30 cm) in September 2016, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Total carbon (Ct) concentration of the soil (% w/w)							
	Fallow	Clover	Field bean	Oat	Maize	<i>p</i> -value	LSD 5% (in % w/w)
No fertilization	1.617 ns	1.634 ns	1.642 ns	1.614 ns	1.498 ns	0.895	0.122
PK+50% N	1.721 ns	1.688 ns	1.730 ns	1.679 ns	1.638 ns	0.148	0.095
PK+100% N	1.586 ns	1.647 ns	1.665 ns	1.710 ns	1.731 ns	0.774	0.212
Mean	1.641	1.656	1.679	1.668	1.622	–	–

The Ct analysis in **2018** was carried out with soil samples taken in September after harvesting the previous crops. It was found that the Ct concentration of the topsoil (0-30 cm) varied from 1.405 to 1.733% w/w (data not shown). The preceding crops had no significant effects on Ct of the topsoil (*p*-value = 0.654) (table 28). In contrary, NPK fertilization has significantly increased the Ct in this soil depth (*p*-value < 0.001) resulting in higher Ct concentration of 1.634% w/w by PK+50% N and 1.678% w/w by the application of PK+100% N. No significant interaction effects by the main factors (pre-crops and NPK fertilization) were observed (*p*-value = 0.979).

In the second depth (30-60 cm), the Ct concentration of the soil was lower and varied from 0.782 to 0.974% w/w (data not shown). In this soil layer neither pre-crops (p -value = 0.435) nor NPK fertilization (p -value = 0.192) have affected the Ct concentration of the soil.

Table 28: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct level of the soil in 2018, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Total carbon (Ct) concentration of the soil (% w/w)			
		September 2018		November 2018	
		0-30 cm	30-60 cm	0-30 cm	30-60 cm
Preceding crop (PC)	Fallow	1.559 ns	0.812 ns	1.555 ns	0.943 ns
	Crimson clover	1.625 ns	0.867 ns	1.663 ns	1.020 ns
	Field bean	1.605 ns	0.840 ns	1.630 ns	0.986 ns
	Oat	1.609 ns	0.815 ns	1.567 ns	0.984 ns
	Maize	1.575 ns	0.870 ns	1.565 ns	1.014 ns
NPK fertilization (NPK)	No fertilization	1.500 c	0.877 ns	1.568 ab	1.020 ns
	PK 50%	1.567 bc	0.859 ns	1.538 b	0.978 ns
	PK+50% N	1.634 ab	0.813 ns	1.636 ab	1.006 ns
	PK+100% N	1.678 a	0.815 ns	1.641 a	0.953 ns
p -value	PC	0.654	0.435	0.067	0.261
	NPK	0.001	0.192	0.027	0.189
	PCxNPK	0.979	0.850	0.957	0.953
LSD 5% (in % w/w)	PC	0.137	0.111	0.124	0.074
	NPK	0.115	0.093	0.104	0.066
	PCxNPK	0.194	0.158	0.176	0.148

In the same year but three months later (in **November 2018**), the 4th Ct analysis was carried out three weeks after incorporating the plant biomass of the pre-crops into the soil. The Ct value of the topsoil varied from 1.480 to 1.732% w/w based on individual plots (data not shown). But no significant effects were found by preceding crops on Ct of the topsoil (p -value = 0.067) (table 28). In contrary, NPK fertilization has significantly increased the Ct concentration of the topsoil (p -value < 0.05). Therefore, PK+100% N (1.641% w/w), PK+50% N (1.636% w/w) and no fertilization (1.568% w/w) achieved the higher Ct values compared to PK 50% (1.538% w/w) (table 28). No significant interaction was found between the main factors (pre-crops and NPK fertilization) (p -value = 0.957). In the depth of 30-60 cm the Ct concentration varied from 0.901 to 1.088% w/w (data not shown). Neither preceding crops (p -value = 0.261) nor NPK fertilization (p -value = 0.189) or interactions between both factors were significantly influenced the Ct levels of the soil (table 28).

The 5th soil analysis was carried out in **March 2019** at juvenile growth of winter wheat. The Ct values of the topsoil (0-30 cm) varied from 1.430 to 1.668% w/w (data not shown). The statistical analysis has shown that neither pre-crops (p -value = 0.462) nor mineral NPK fertilization (p -value = 0.276) significantly affected the Ct of the soil (table 29). Furthermore, no significant interaction effects were found by the main factors (Pre-crops and NPK fertilization) on Ct of the topsoil (p -value = 0.524).

In the second depth (30-60 cm) the same trend was found compared to 0-30 cm. The Ct concentration of the soil varied from 0.922 to 1.137% w/w among the treatments (data not shown). The Ct value was not influenced by either preceding crops (p -value = 0.675) or NPK fertilization (p -value = 0.903). In addition, no significant interaction effects were found by the main factors (Pre-crops and NPK fertilization) (p -value = 0.209) (table 29).

The Ct level of the subsoil in 60-90 cm was lower compared to the upper horizons and varied from 0.612 to 0.865% w/w (data not shown). Based on the analysed data, the Ct concentration of this soil layer were neither affected by preceding crops (p -value = 0.738) nor by NPK fertilization (p -value = 0.296) and no interaction was between both main factors (pre-crops and NPK fertilization) was observed (p -value = 0.957).

Table 29: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct concentration of the soil in 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Total carbon (Ct) concentration of the soil (% w/w)					
		March 2019			October 2019		
		0-30 cm	30-60 cm	60-90 cm	0-30 cm	30-60 cm	60-90 cm
PC	Fallow	1.528 ns	1.075 ns	0.748 ns	1.560 ab	1.133 ns	0.687 ns
	Crimson clover	1.535 ns	1.025 ns	0.703 ns	1.629 a	1.176 ns	0.727 ns
	Field bean	1.543 ns	1.041 ns	0.674 ns	1.617 a	1.141 ns	0.720 ns
	Oat	1.577 ns	0.998 ns	0.716 ns	1.585 a	1.134 ns	0.711 ns
	Maize	1.604 ns	1.006 ns	0.700 ns	1.501 b	1.109 ns	0.786 ns
NPK	No fertilization	1.562 ns	1.009 ns	0.758 ns	1.580 ab	1.208 a	0.738 ns
	PK 50%	1.521 ns	1.042 ns	0.717 ns	1.522 b	1.070 b	0.696 ns
	PK+50% N	1.602 ns	1.042 ns	0.682 ns	1.623 a	1.188 a	0.711 ns
	PK+100% N	1.543 ns	1.023 ns	0.675 ns	1.589 ab	1.087 b	0.760 ns
p -value	PC	0.462	0.675	0.738	0.013	0.634	0.476
	NPK	0.276	0.903	0.296	0.038	< 0.001	0.572
	PCxNPK	0.524	0.209	0.957	0.902	0.995	0.958
LSD 5% (in % w/w)	PC	0.095	0.114	0.108	0.077	0.085	0.110
	NPK	0.085	0.102	0.097	0.069	0.076	0.098
	PCxNPK	0.189	0.228	0.216	0.155	0.170	0.219

One year after cultivating of pre-crops the 6th soil analysis was carried out in **October 2019**, which has shown Ct variation in 0-30 cm from 1.460 to 1.680% w/w among the treatments (data not shown). Significant effects of preceding crops on Ct of the topsoil were observed (p -value < 0.05) resulting in higher Ct concentrations in the soil after crimson clover (1.629% w/w), field bean (1.617% w/w), oat (1.585% w/w) and fallow (1.560% w/w) compared to maize (1.501% w/w) (table 29). Furthermore, the Ct of the topsoil was also significantly influenced by NPK fertilization (p -value < 0.05) resulting in higher Ct in PK+100% N (1.589% w/w), PK+50% N (1.623% w/w) and no fertilization (1.580% w/w) compared to PK 50% (1.522% w/w). However, no significant interaction effects were found by the main factors (pre-crops and NPK fertilization) (p -value = 0.902).

In the second depth (30-60 cm), the Ct variation of the soil was from 1.033 to 1.227% w/w (data not shown). No significant effects on Ct of the soil were found by preceding crops (p -

value = 0.634). In contrary, mineral NPK fertilization has significantly affected the Ct of the soil (p -value < 0.001). PK+50% N (1.188% w/w) and no fertilization (1.208% w/w) led to the highest Ct value compared to PK 50% (1.070% w/w) and PK+100% N (1.087% w/w) (table 29).

In the third depth (60-90 cm), the Ct value of the subsoil varied from 0.662 to 0.913% w/w among the treatments (data not shown). Neither preceding crops (p -value = 0.476) nor mineral NPK fertilization (p -value = 0.572) was influenced the Ct value of the subsoil. Therefore, no significant interaction effects were found by the main factors (pre-crops and NPK fertilization) (p -value = 0.958).

The seventh and last Ct analysis was carried out in **April 2020**, which shows Ct variation of the soil (0-30 cm) from 1.440 to 1.715% w/w among treatments (data not shown). Crimson clover (1.654% w/w), field bean (1.582% w/w) and fallow (1.580% w/w) significantly increased the Ct concentration of the topsoil (0-30 cm) (p -value = 0.047) compared to oat (1.544% w/w) and maize (1.547% w/w) (table 30). Furthermore, the Ct positively responded to NPK fertilization (p -value < 0.001) resulting in higher Ct in PK+100% N (1.618% w/w) and PK+50% N (1.650% w/w) of the topsoil compared to no fertilization (1.539% w/w). No significant interaction effects were found by the main factors (pre-crops and NPK fertilization) (p -value = 0.829).

In the second soil depth (30-60 cm), the Ct level of the soil has varied from 0.977 to 1.140% w/w between the treatments (data not shown). Neither pre-crops (p -value = 0.202) nor NPK fertilization (p -value = 0.455) or interaction between both main factors on the Ct level of the soil was observed (table 30). In the deepest soil layer (60-90 cm) the same tendency was found like in the second depth (30-60 cm). The Ct level of the subsoil varied from 0.588 to 0.765% w/w (data not shown) but not significant effects by pre-crops (p -value = 0.421) or NPK fertilization (p -value = 0.597) were found (table 30). No interaction between both factors occurred in this study either (p -value = 0.816).

Table 30: Effect of preceding crops (PC) and NPK fertilization (NPK) on Ct level of the soil in April 2020, LTE "BNF" Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Total carbon (Ct) concentration of the soil (% w/w)		
		0-30 cm	30-60 cm	60-90 cm
Preceding crop (PC)	Fallow	1.580 ab	1.025 ns	0.668 ns
	Crimson clover	1.654 a	1.103 ns	0.700 ns
	Field bean	1.582 ab	1.054 ns	0.673 ns
	Oat	1.544 b	1.079 ns	0.675 ns
	Maize	1.547 b	1.111 ns	0.733 ns
NPK fertilization (NPK)	No fertilization	1.539 b	1.097 ns	0.714 ns
	PK 50%	1.517 b	1.046 ns	0.670 ns
	PK+50% N	1.650 a	1.091 ns	0.681 ns
	PK+100% N	1.618 a	1.062 ns	0.695 ns
p -value	PC	0.047	0.202	0.421
	NPK	< 0.001	0.455	0.597
	PCxNPK	0.829	0.956	0.816
LSD 5% (in % w/w)	PC	0.078	0.081	0.077
	NPK	0.070	0.073	0.069
	PCxNPK	0.157	0.163	0.153

4. 1. 7 Dissolved organic carbon (DOC)

The DOC analysis was carried out with soil samples taken in **September 2018**, two weeks after harvesting the last preceding crop. The DOC in the first layer of the soil (0-30 cm) varied from 39 to 102 kg ha⁻¹ (figure 18). The statistical analysis of DOC data revealed significant interaction between the main factors pre-crops and NPK fertilization (p -value = 0.031). Therefore, using the optimal dosage of mineral NPK fertilization (PK+100% N) led crimson clover to increase the DOC of the topsoil to the maximum level of 102 kg ha⁻¹.

Based on the analysed data (table 31), DOC of the topsoil (0-30 cm) was not significantly influenced by pre-crops (p -value = 0.127) but only with mineral NPK fertilization (p -value < 0.001). The DOC level of the topsoil (0-30 cm) was slightly higher under crimson clover (66 kg ha⁻¹) and oat (62.6 kg ha⁻¹) compared to field bean (59.4 kg ha⁻¹), maize (58.7 kg ha⁻¹) and fallow (51.4 kg ha⁻¹), however, no significant effect were found between different pre-crops.

In addition, the optimal dosage of NPK fertilization (PK+100% N) significantly increased the DOC of the soil to the level of 76.4 kg ha⁻¹, whereas PK+50% N (55.8 kg ha⁻¹) and PK 50% (60.1 kg ha⁻¹) obtained the same levels and no fertilization (46.2 kg ha⁻¹) dedicated the lower level of DOC in the topsoil (table 31).

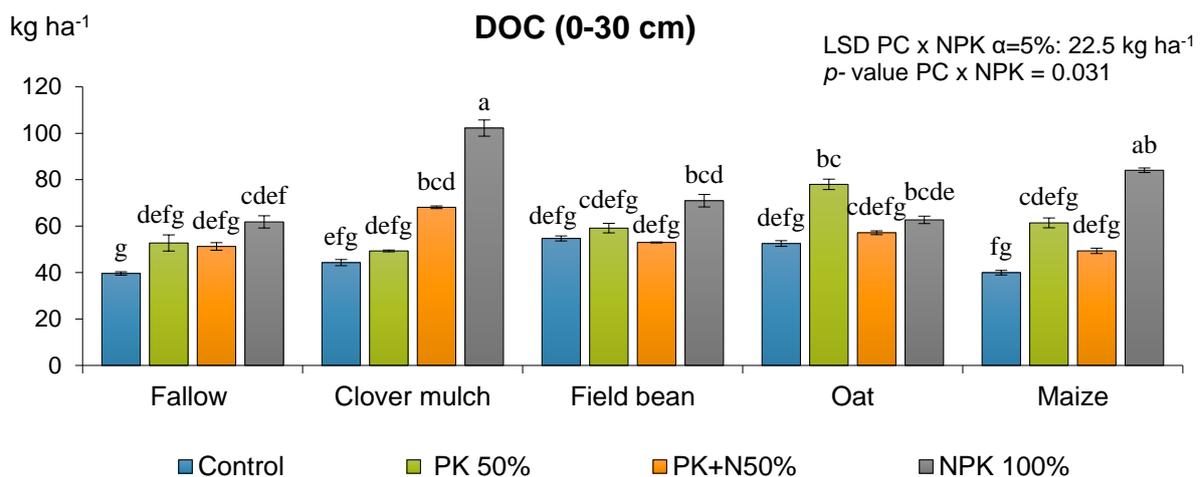


Figure 18: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on DOC of the topsoil (0-30 cm) September 2018, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

In the second depth (30-60 cm), the DOC of the soil varied from 22.3 to 36.1 kg ha⁻¹ (data not shown). Neither preceding crops (p -value = 0.617) nor mineral NPK fertilization (p -value = 0.965) were influenced the DOC of the soil (table 31). Furthermore, no significant interaction effects were found between the main factors (pre-crops and mineral NPK fertilization) (p -value = 0.940).

Table 31: Effect of preceding crops (PC) and NPK fertilization (NPK) on DOC of the soil (September 2018), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Dissolved organic carbon (DOC) of the soil (kg ha ⁻¹)	
		September 2018	
		0-30 cm	30-60 cm
Preceding crop (PC)	Fallow	51.4 ns	27.3 ns
	Crimson clover	66.0 ns	32.4 ns
	Field bean	59.4 ns	27.9 ns
	Oat	62.6 ns	25.7 ns
	Maize	58.7 ns	26.1 ns
NPK fertilization (NPK)	No fertilization	46.2 c	28.8 ns
	PK 50%	60.1 b	28.2 ns
	PK+50% N	55.8 bc	26.7 ns
	PK+100% N	76.4 a	27.8 ns
<i>p</i> -value	PC	0.127	0.617
	NPK	< 0.001	0.965
	PCxNPK	0.031	0.940
LSD 5% (kg ha ⁻¹)	PC	11.3	9.3
	NPK	10.1	8.3
	PCxNPK	22.5	18.5

4. 1. 8 Total organic carbon (TOC)

The TOC was determined in soil samples taken in **November 2018**, three weeks after incorporating the plant biomass of the pre-crops into the soil. The TOC values of the topsoil varied from 13 to 26 mg/l (table 32). The analysis of variance has shown significant effects on TOC of the topsoil (0-30 cm) caused by preceding crops (*p*-value < 0.001) as well as by mineral NPK fertilization (*p*-value < 0.001). However, crimson clover (22 mg/l), field bean (20 mg/l), oat (21 mg/l) and maize (19 mg/l) significantly increased the TOC of the topsoil compared to fallow (16 mg/l). In addition, PK+50% N (21.5 mg/l), PK+100% N (20.6 mg/l) and no fertilization (20.1 mg/l) achieved higher values of TOC compared to PK 50% (16.8 mg/l). No significant interaction effects were found between the main factors (Pre-crops and NPK fertilization) (*p*-value = 0.139).

Table 32: Effect of preceding crops (PC) and NPK fertilization (NPK) on TOC of the topsoil (0-30 cm) November 2018, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments.

Total organic carbon (TOC) of the topsoil (0-30 cm) in mg/l					
Treatment	No fertilization	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	16.7 ns	12.9 ns	18.0 ns	16.6 ns	16.0 b
Crimson clover	20.5 ns	19.3 ns	26.2 ns	21.7 ns	21.9 a
Field bean	22.0 ns	15.8 ns	21.0 ns	22.7 ns	20.4 a
Oat	21.6 ns	17.8 ns	19.0 ns	25.2 ns	20.9 a
Maize	19.6 ns	18.0 ns	23.1 ns	16.7 ns	19.4 a
Mean	20.1 a	16.8 b	21.5 a	20.6 a	19.7
<i>p</i> -value	PC: < 0.001		NPK: < 0.001		PC x NPK: 0.139
LSD 5%	PC: 2.6 mg/l		NPK: 2.3 mg/l		PC x NPK: 5.3 mg/l

The second TOC analysis was carried with soil samples from **March 2019** at juvenile growth of winter wheat. The TOC value of the topsoil (0-30 cm) varied from 14.2 to 22 mg/l (data not shown). Based on the analysed data (table 33) the TOC of the topsoil was significantly affected by preceding crops (p -value < 0.001) and mineral NPK fertilization (p -value < 0.001). No significant interaction effects were found between both main factors (pre-crops and NPK fertilization) (p -value = 0.619). Crimson clover (19.3 mg/l), oat (18.3 mg/l) and maize (18.3 mg/l) have achieved the highest TOC of the soil compared to field bean (16.9 mg/l) and fallow (15.5 mg/l). In addition, the higher dosage of mineral fertilization (PK+100% N) has increased the TOC value of the soil to 20 mg/l compared to PK+50% N (17.6 mg/l), PK 50% (17.1 mg/l) and no fertilization (16.1 mg/l).

The TOC value of the second depth (30-60 cm) varied from 11.5 to 15.7 mg/l (data not shown). The TOC of the soil (30-60 cm) was influenced only by preceding crops (p -value < 0.001), but not by mineral NPK fertilization (p -value = 0.787) (table 33). Again, no significant interaction effects were found between pre-crops and NPK fertilization (p -value = 0.593). Based on the statistical analysis, crimson clover (14.5 mg/l), maize (14.3 mg/l), field bean (13.3 mg/l) and oat (13.2 mg/l) have steady increased the TOC value of the soil compared to fallow (11.8 mg/l).

A further TOC analysis was carried out with soil samples taken from the subsoil (60-90 cm). In this soil layer the TOC varied from 6.8 to 13.2 mg/l (data not shown) and was not influenced by preceding crops (p -value = 0.706) but by mineral NPK fertilization (p -value = 0.014). It was observed that in no fertilization (11.2 mg/l) and PK+100% N (9.6 mg/l) the highest TOC values of the subsoil was obtained compared to PK+50% N (8.8 mg/l) and PK 50% (8.2 mg/l) (table 33).

Table 33: Effect of preceding crops (PC) and NPK fertilization (NPK) on TOC concentration of the soil in March 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Treatments		Total Organic Carbon (TOC) of the soil (mg/l)		
		March 2019		
		0-30 cm	30-60 cm	60-90 cm
Preceding crop (PC)	Fallow	15.5 c	11.8 d	8.6 ns
	Crimson clover	19.3 a	14.5 a	9.8 ns
	Field bean	16.9 bc	13.3 bc	9.5 ns
	Oat	18.3 ab	13.2 c	9.3 ns
	Maize	18.3 ab	14.3 ab	10.0 ns
NPK fertilization (NPK)	No fertilization	16.1 b	13.3 ns	11.2 a
	PK 50%	17.1 b	13.8 ns	8.8 b
	PK+50% N	17.6 b	13.4 ns	8.2 b
	PK+100% N	19.9 a	13.3 ns	9.6 ab
p -value	PC	< 0.001	< 0.001	0.706
	NPK	< 0.001	0.787	0.014
	PC×NPK	0.619	0.593	0.840
LSD 5%	PC	1.8 mg/l	1.3 mg/l	2.1 mg/l
	NPK	1.6 mg/l	1.2 mg/l	1.8 mg/l
	PC×NPK	3.6 mg/l	2.6 mg/l	4.1 mg/l

4. 1. 9 Cation Exchange Capacity (CEC)

In this study the CEC of the topsoil (0-30 cm) was determined only in those samples taken in **March 2019** since it is assumed that the CEC of the soil only change over long periods of time, so that a single CEC determination of the samples of this experiment is sufficient. It was found that the CEC of the soil varied from 16.2 to 18.1 meq/100 g soil (table 34). Based on analysed data the CEC of the topsoil was influenced only by mineral NPK fertilization (p -value < 0.001) but not by preceding crops (p -value = 0.236). Furthermore, no interaction effects were found between both test factors (pre-crops and NPK fertilization) (p -value = 0.792). Based on the analysed data, a full dosage of mineral NPK fertilization (PK+100% N) has increased the CEC of the soil (17.5 meq/100 g soil) slightly but significant compared to the no fertilization (16.5 meq/100 g soil).

Table 34: Effect of preceding crops (PC) and NPK fertilization (NPK) on CEC of the topsoil (0-30 cm) in March 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Cation Exchange Capacity (CEC) in the soil (meq/100 g soil)						
Treatments	Fallow	Clover	Field bean	Oat	Maize	Mean
No fertilization	16.3 ns	16.5 ns	16.9 ns	16.8 ns	16.2 ns	16.5 b
PK+100% N	17.3 ns	16.7 ns	17.8 ns	18.1 ns	17.4 ns	17.5 a
Mean	16.8 ns	16.6 ns	17.3 ns	17.5 ns	16.8 ns	17.0
p -value	PC: 0.236		NPK: 0.002		PCxNPK: 0.792	
LSD 5% (meq/100 g)	PC: 0.9		NPK: 0.6		PCxNPK: 1.2	

4. 1. 10 Soil microbial biomass carbon (SMB C)

As part of the investigations carried out, the soil of the experiment was examined for the soil microbial biomass C (SMB C) on a total of five different dates between September 2016 and October 2020, once in the 9th crop rotation and four times in the 10th crop rotation. These investigations were intended to obtain representative results about the influence of the treatments and possible changes within the crop rotation. It was expected to get a trend over time of the effect of management on soil microorganisms. The detailed results of the five dates are presented below.

The first soil microbial biomass C analysis of the soil (in 0-30 cm) was carried out in **September 2016** (9th crop rotation), three weeks after harvesting winter rye and two years after the pre-crops. In that year the SMB C of the soil (0-30 cm) varied from 250 to 344 $\mu\text{g C/g DW}$ (table 35). Based on the statistical analysis no clear effect by the preceding crops (p -value = 0.196) or mineral NPK fertilization (p -value = 0.626) nor an interaction between both factors (p -value = 0.190) was found (table 35).

Table 35: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-30 cm) in September 2016, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Soil microbial biomass carbon (SMB C) of the topsoil (0-30 cm) in µg C/g DW				
Treatments	No fertilization	PK+50% N	PK+100% N	Mean
Fallow	315 ns	329 ns	250 ns	298 ns
Crimson clover	306 ns	341 ns	344 ns	331 ns
Field bean	303 ns	344 ns	316 ns	321 ns
Oat	329 ns	323 ns	319 ns	324 ns
Maize	286 ns	269 ns	322 ns	293 ns
Mean	308 ns	321 ns	310 ns	313 ns
p-value	PC: 0.196	NPK: 0.626	PC×NPK: 0.190	
LSD 5% (µg C/g DW)	PC: 38	NPK: 29	PC×NPK: 66	

The 2nd SMB C analysis of the soil was carried out two years later in **September 2018** (10th crop rotation), with soil samples taken two weeks after harvesting pre-crops. In this year the level of the soil microbial biomass C of the soil (0-30 cm) was quite low and varied from 156 to 259 µg C/g DW (table 36). In this investigation a significant effect of preceding crops on the soil microbial biomass C of the soil was found (p -value < 0.001). The treatments fallow (223 µg C/g DW), oat (210 µg C/g DW) and crimson clover (193 µg C/g DW) had the same SMB C level which was higher compared to field bean (184 µg C/g DW) and maize (161 µg C/g DW). No significant effect of mineral NPK fertilization (p -value = 0.100) and no interaction effects were found between the main factors (pre-crops and mineral NPK fertilization) (p -value = 0.471).

Table 36: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-30 cm) in September 2018, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Soil microbial biomass carbon (SMB C) of the topsoil (0-30 cm) in µg C/g DW					
Treatments	No fertilization	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	201 ns	207 ns	247 ns	237 ns	223 a
Crimson clover	190 ns	182 ns	186 ns	214 ns	193 abc
Field bean	175 ns	172 ns	208 ns	183 ns	184 bc
Oat	195 ns	195 ns	189 ns	259 ns	210 ab
Maize	171 ns	158 ns	159 ns	156 ns	161 c
Mean	186 ns	183 ns	198 ns	210 ns	194
p-value	PC: < 0.001	NPK: 0.100		PC×NPK: 0.471	
LSD 5% (µg C/g DW)	PC: 26	NPK: 23		PC×NPK: 53	

The third determination of the soil microbial biomass C of the soil (0-30 cm) was carried out in **October 2019**, one year after cultivating pre-crops. In that year, the SMB C of the soil was characterized by higher variation from minimum 248 to maximum 401 µg C/g DW (table 37). Based on the analysed data, significant effects were found by preceding crops (p -value <

0.001) as well as by mineral NPK fertilization (p -value < 0.001). Crimson clover (346 $\mu\text{g C/g DW}$) and oat (349 $\mu\text{g C/g DW}$) significantly increased the SMB C of the soil compared to field bean (308 $\mu\text{g C/g DW}$) and fallow (267 $\mu\text{g C/g DW}$). In addition, PK+100% N (335 $\mu\text{g C/g DW}$) and PK+50% N (344 $\mu\text{g C/g DW}$) led to the highest level of SMB C of the topsoil compared to PK 50% (282 $\mu\text{g C/g DW}$) and no fertilization (297 $\mu\text{g C/g DW}$).

Table 37: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-30 cm) in October 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Soil microbial biomass carbon (SMB C) of the topsoil (0-30 cm) in $\mu\text{g C/g DW}$					
Treatments	No fertilization	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	266 ns	248 ns	261 ns	295 ns	267 c
Crimson clover	308 ns	325 ns	370 ns	382 ns	346 a
Field bean	291 ns	251 ns	352 ns	339 ns	308 b
Oat	320 ns	281 ns	401 ns	394 ns	349 a
Maize	300 ns	307 ns	337 ns	268 ns	303 b
Mean	297 b	282 b	344 a	335 a	315
p -value	PC: < 0.001		NPK: < 0.001		PC×NPK: 0.130
LSD 5% ($\mu\text{g C/g DW}$)	PC: 34		NPK: 30		PC×NPK: 68

The fourth SMB C analysis of the soil (0-30 cm) was carried out in **April 2020** two years after the preceding crops resulting in a slight variation from 216 to 320 $\mu\text{g C/g DW}$ (table 38). Based on the statistical analysis of the data no significant effects were found neither by pre-crops (p -value = 0.140) nor by mineral NPK fertilization (p -value = 0.053) or by the interaction between both factors (p -value = 0.683).

Table 38: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-30 cm) in April 2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Soil microbial biomass carbon (SMB C) of the topsoil (0-30 cm) in $\mu\text{g C/g DW}$					
Treatments	No fertilization	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	240 ns	215 ns	289 ns	247 ns	248 ns
Crimson clover	216 ns	308 ns	300 ns	320 ns	286 ns
Field bean	235 ns	230 ns	276 ns	259 ns	250 ns
Oat	245 ns	225 ns	276 ns	303 ns	262 ns
Maize	287 ns	288 ns	304 ns	278 ns	289 ns
Mean	245 ns	253 ns	289 ns	281 ns	267
p -value	PC: 0.140		NPK: 0.053		PC×NPK: 0.683
LSD 5% ($\mu\text{g C/g DW}$)	PC: 41		NPK: 37		PC×NPK: 83

The fifth determination of the soil microbial biomass C was carried out in **October 2020** in the two depths 0-15 and 15-30 cm. It was found that the SMB C of the top layer (0-15 cm) varied from 426 to 612 $\mu\text{g C/g DW}$ whereas, in the deeper soil layer (15-30 cm) there was a greatly variation from minimum 213 to maximum 649 $\mu\text{g C/g DW}$ (table 39). Despite the large

variation of the soil microbial biomass C in both soil layers, no significant effects were observed between the main factors (pre-crops and NPK fertilization) (table 39).

When looking at the mean values of the pre crops variants in both depths, the following ranking becomes visible: in 0-15 cm crimson clover has reached the highest and follow the lowest values and in 15 – 30 cm field bean caused the highest and oat had the lowest SMB C.

Table 39: Effect of preceding crops (PC) and NPK fertilization (NPK) on SMB C of the topsoil (0-15 and 15-30 cm) in October 2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Soil microbial biomass carbon (SMB C) of the topsoil in $\mu\text{g C/g DW}$					
0-15 cm					
Treatments	No fertilization	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	471 ns	439 ns	481 ns	490 ns	470 ns
Crimson clover	586 ns	461 ns	537 ns	612 ns	549 ns
Field bean	479 ns	426 ns	528 ns	532 ns	491 ns
Oat	430 ns	512 ns	513 ns	538 ns	498 ns
Maize	488 ns	504 ns	519 ns	482 ns	498 ns
Mean	491 ns	468 ns	516 ns	531 ns	501
<i>p</i> -value	PC: 0.183		NPK: 0.157		PCxNPK: 0.716
LSD 5% ($\mu\text{g C/g DW}$)	PC: 65		NPK: 58		PCxNPK: 129
15-30 cm					
Treatments	No fertilization	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	451 ns	404 ns	411 ns	325 ns	398 ns
Crimson clover	343 ns	500 ns	421 ns	587 ns	463 ns
Field bean	464 ns	450 ns	491 ns	649 ns	513 ns
Oat	213 ns	364 ns	307 ns	429 ns	328 ns
Maize	363 ns	313 ns	521 ns	401 ns	400 ns
Mean	367 ns	406 ns	430 ns	478 ns	420
<i>p</i> -value	PC: 0.114		NPK: 0.374		PCxNPK: 0.765
LSD 5% ($\mu\text{g C/g DW}$)	PC: 143		NPK: 128		PCxNPK: 286

4. 1. 11 Soil fauna feeding activity

The soil fauna feeding activity based on bait strips inserted vertically into the soil surface (0-10 cm), was quantitative determined in August 2020 in the 10th crop rotation, two months after harvesting winter rye. The received data have shown a quite large variation of the feeding activity ranging from 36.6 to 72.8 % (table 40). Based on analysed data the feeding activity was significantly influenced by preceding crops (*p*-value < 0.001) as well as mineral fertilization (*p*-value = 0.018). However, no significant interaction effects were observed between both factors (pre-crops and NPK fertilization) (*p*-value = 0.877). The treatment with crimson clover mulch (62.6 %) led to significant increase of the soil fauna feeding activity compared to oat (49.2 %) and fallow (51.4 %). In addition, lower feeding activity was found by mineral NPK fertilization (51.4 %) compared to No fertilization (57.3 %).

Table 40: Effect of preceding crops (PC) and NPK fertilization (NPK) on soil fauna feeding activity in August 2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatments, ns: not significant.

Soil fauna feeding activity (bait strips) (%) – August 2020					
Treatments		Preceding crop			
NPK fertilization	Field Rep.	Fallow	Crimson clover	Oat	Mean
No fertilization	1	53.9 ns	72.8 ns	44.8 ns	57.3 a
	2	52.4 ns	64.4 ns	56.0 ns	
	3	58.3 ns	68.9 ns	58.9 ns	
	4	50.2 ns	58.8 ns	48.5 ns	
PK+100% N	1	36.6 ns	49.1 ns	38.8 ns	51.4 b
	2	56.3 ns	69.2 ns	47.5 ns	
	3	55.1 ns	64.4 ns	57.7 ns	
	4	48.5 ns	52.8 ns	41.3 ns	
Mean		51.4 b	62.6 a	49.2 b	
<i>p</i> -value		PC: < 0.001	NPK: 0.018	PCxNPK: 0.877	
LSD 5%		PC: 5.8%	NPK: 4.7%	PCxNPK: 8.2%	

4. 1. 12 Mesofauna (micro arthropods) of the soil

The number of the intermediate-sized animals (0.2 to 4 mm length) in the soil also called as micro-arthropods were detected in August 2020 at LTE “BNF” experimental station Weilburger Grenze. In total 16 groups of soil arthropods including: collembola, mites, pauropoda, symphyla, pseudoscorpions, chilopoda, protura, diplopoda, diplura, isopoda, coleoptera, spiders, worms (e. g. wireworms, elateridae), larvae, earth bees and ants were identified within the sixty soil samples (0-15 cm) which were analyzed. Based on the analyzed data, mites with 79% made up the majority of the soil micro arthropods followed by collembola with 14%, pauropods (pauropoda) with 2% and others with 5% (figure 19).

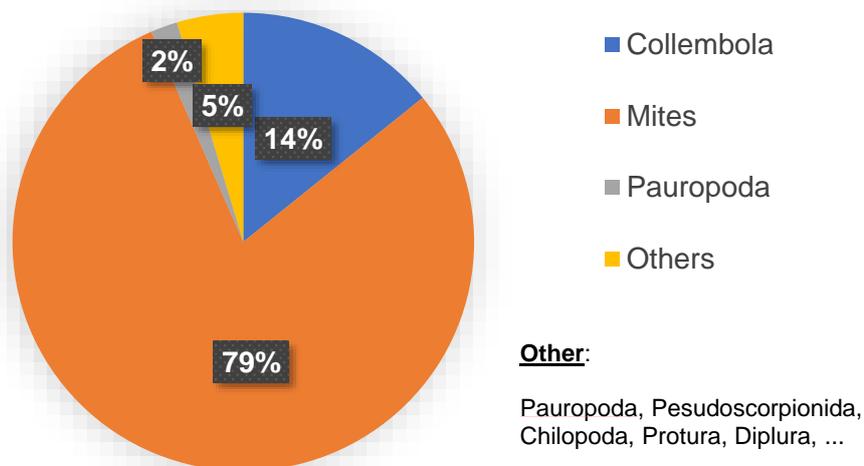


Figure 19: Mesofauna abundance (in %) in the soil (0-15 cm) in the LTE “BNF”, soil samples from August 2020, experimental station “Weilburger Grenze” Gießen.

The remaining 13 groups of micro arthropods were observed only sporadically and therefore only accounted for 5% of the total population. Representative examples of the morphology and phenotypic appearance of the individual species or groups that have been identified are shown in figure 20.

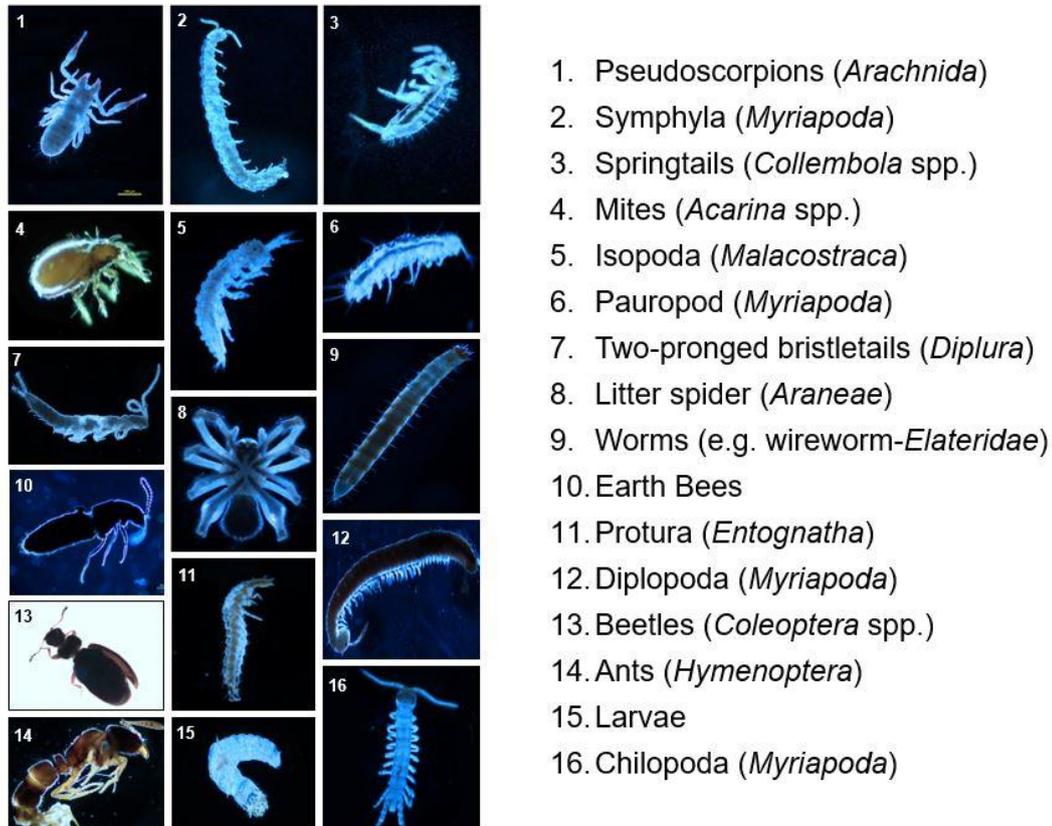


Figure 20: Micro arthropods (16 groups) identified in the soil (0-10 cm) in LTE “BNF” in August 2020, experimental station “Weilburger Grenze” Gießen, own photos (microscope Nikon Eclipse E600, lens scale 4X, 10X and 20X).

Springtails (*Collembola* spp.)

The number of springtails in the soil varied between the treatments from 14 to 29 (table 40). Based on statistical analysis the population significantly responded to mineral NPK fertilization (p value = 0.007) (table 41). It could be observed that the optimal dosage of mineral NPK fertilization (PK+100% N) has increased the number of springtails to 25 compared to the No fertilization in which only 15 springtails were counted. In addition, no significant effect of pre-crops on springtails population was found.

Mites (*Acarina* spp.)

Mites made up by far the largest proportion of the soil micro-arthropods population. The number of individuals varied greatly from minimum 85 to maximum 196 (table 41). The analysis of variance revealed a significant pre-crop effect on mites population of the soil (p value = 0.045). Clover mulch has increased the number of mites to the maximum level of 159 compared to oat (89) and fallow (91) which caused lower values. No significant effects were found by mineral NPK fertilization on Mites population (table 41).

Pauropoda (Myriapods) and others

The population density of *Pauropoda* which was analysed in the soil samples was very low (<5) (table 41). Based on analysed data neither pre-crops (p value = 0.355) nor mineral NPK fertilization (p value ≥ 1) have influenced the population density of *Pauropoda* in the soil (table 41). The remaining 13 arthropod groups made up a relative proportion of 5% and were therefore of very little contribution on the trial soil area. The abundance of these groups/species was not influenced by neither pre-crops (p value = 0.667) nor mineral NPK fertilization (p value = 0.134) (table 41).

Table 41: Number of micro arthropods depending on pre-crops (PC) and NPK fertilization (NPK) in the soil, LTE “BNF” Weilburger Grenze Gießen 2020.

Treatments		No. of groups	Number of detected micro arthropods				
Pre-crop	NPK		Total	Collembola	Mites	Pauropoda	Others
Fallow	No NPK	16	113 ^{ns}	14 ^{ns}	93 ^b	1 ^{ns}	4 ^{ns}
Oat		16	111 ^{ns}	17 ^{ns}	85 ^b	4 ^{ns}	7 ^{ns}
Clover		16	217 ^{ns}	14 ^{ns}	196 ^a	3 ^{ns}	6 ^{ns}
Mean (No NPK)			147 ^{ns}	15 ^b	125 ^{ns}	3 ^{ns}	6 ^{ns}
Fallow	100% NPK	16	127 ^{ns}	29 ^{ns}	89 ^b	2 ^{ns}	8 ^{ns}
Oat		16	126 ^{ns}	24 ^{ns}	93 ^b	4 ^{ns}	8 ^{ns}
Clover		16	153 ^{ns}	22 ^{ns}	121 ^a	2 ^{ns}	7 ^{ns}
Mean (100% NPK)			135 ^{ns}	25 ^a	101 ^{ns}	3 ^{ns}	8 ^{ns}
p -value	PC		0.056	0.702	0.045	0.355	0.667
	NPK		0.638	0.007	0.329	1.000	0.134
	PC x NPK		0.314	0.667	0.329	0.873	0.545
LSD 5%	PC		61	8	61	3	3
	NPK		50	7	50	2	2
	PC x NPK		87	12	87	4	4

4. 1. 13 Soil surface CO₂ efflux (Fc) dynamic

Treatment and seasonal effect of CO₂ efflux of the soil surface

Soil surface CO₂ efflux dynamic was determined every week in two consecutive growing seasons from **autumn 2018** to **winter 2020** within 10th crop rotation of the LTE “BNF”. Based on the analysed data (table 42) of sixteen individual observations during the **growing season 2018/19**, the near surface Fc dynamic (min. – max.) of the soil slightly varied between the treatments. In autumn 2018, clover (13.8 – 31.3 $\mu\text{g C m}^{-2} \text{s}^{-1}$) has led to the same Fc level as oat (13.6 – 28.6 $\mu\text{g C m}^{-2} \text{s}^{-1}$) (table 42). The following measurements revealed that Fc values were slightly higher in summer 2019 (in winter wheat) compared to autumn 2018. Hence, crimson clover (38.2 – 63.0 $\mu\text{g C m}^{-2} \text{s}^{-1}$), oat (42.8 – 77.5 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and fallow (25.2 – 69.6 $\mu\text{g C m}^{-2} \text{s}^{-1}$) used as preceding crops have achieved higher Fc variation from April to July 2019 (table 42). However, no clear effects were found by preceding crops or mineral NPK fertilization nor by the interaction effects between the main factors (table A. 1).

Table 42: Effect of preceding crops (PC) and NPK fertilization (NPK) on CO₂ efflux (Fc) of the soil surface in autumn 2018 and spring / summer 2019 during wheat growth cycle, LTE “BNF” Giessen (Obs.: no. of observations, SE: standard error).

CO ₂ efflux (Fc) of the soil ($\mu\text{g C m}^{-2} \text{s}^{-1}$) in winter wheat										
Treatments	Autumn					Spring / Summer				
	14.11. – 17.12.2018					16.04. – 27.07.2019				
Pre-crops	Obs.	Min.	Max.	Mean	SE	Obs.	Min.	Max.	Mean	SE
Fallow	0	–	–	–	–	11	25.2	69.6	47.0	1.9
Clover	5	13.8	31.3	20.2	2.4	11	38.2	63.0	51.2	2.2
Oat	5	13.6	28.6	20.5	2.3	11	42.8	77.5	57.1	2.4
NPK fertilization										
No NPK	0	–	–	–	–	11	34.7	75.0	54.4	2.5
PK+50% N	5	13.7	29.6	20.4	2.7	0	–	–	–	–
PK+100% N	0	–	–	–	–	11	36.6	57.4	48.8	1.8

The Fc measurement campaign started in November 2018 on plots after clover mulch and oat (figure 21). On 14th and 26th of November clover mulch (22.2 and 31.3 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and oat (23.9 and 28.6 $\mu\text{g C m}^{-2} \text{s}^{-1}$) recorded steady the same variations, respectively. This trend was declined by frost on 3rd, 10th and 17th of December in the treatment after clover (14.6, 18.8 and 13.8 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and oat (19.9, 16.8 and 13.6 $\mu\text{g C m}^{-2} \text{s}^{-1}$).

The Fc varied depending on air/soil temperature and soil moisture. Within the whole measurement campaign from autumn 2018 to autumn 2020, the near surface CO₂ efflux in the cooler seasons was recorded at minimum level of 14 $\mu\text{g C m}^{-2} \text{s}^{-1}$ (autumn 2018 and spring 2019) (figure 21). The values were even lower in autumn 2020, when the efflux rate dropped to 4 $\mu\text{g C m}^{-2} \text{s}^{-1}$ due to the occurrence of frost. On the other hand, the maximum rate of CO₂ efflux was observed in summer 2019 with 77 $\mu\text{g C m}^{-2} \text{s}^{-1}$ and, also in summer 2020 with 76 $\mu\text{g C m}^{-2} \text{s}^{-1}$ after a period of high air temperature and drought (figure 21). In 2020, the Fc dynamic was higher in summer (June – August) caused by increasing the air temperature (13 – 24 °C) and the soil moisture of (0.09 $\text{m}^3 \text{m}^{-3}$) compared to spring (April-May) (7.6 – 17 °C and 0.16 $\text{m}^3 \text{m}^{-3}$).

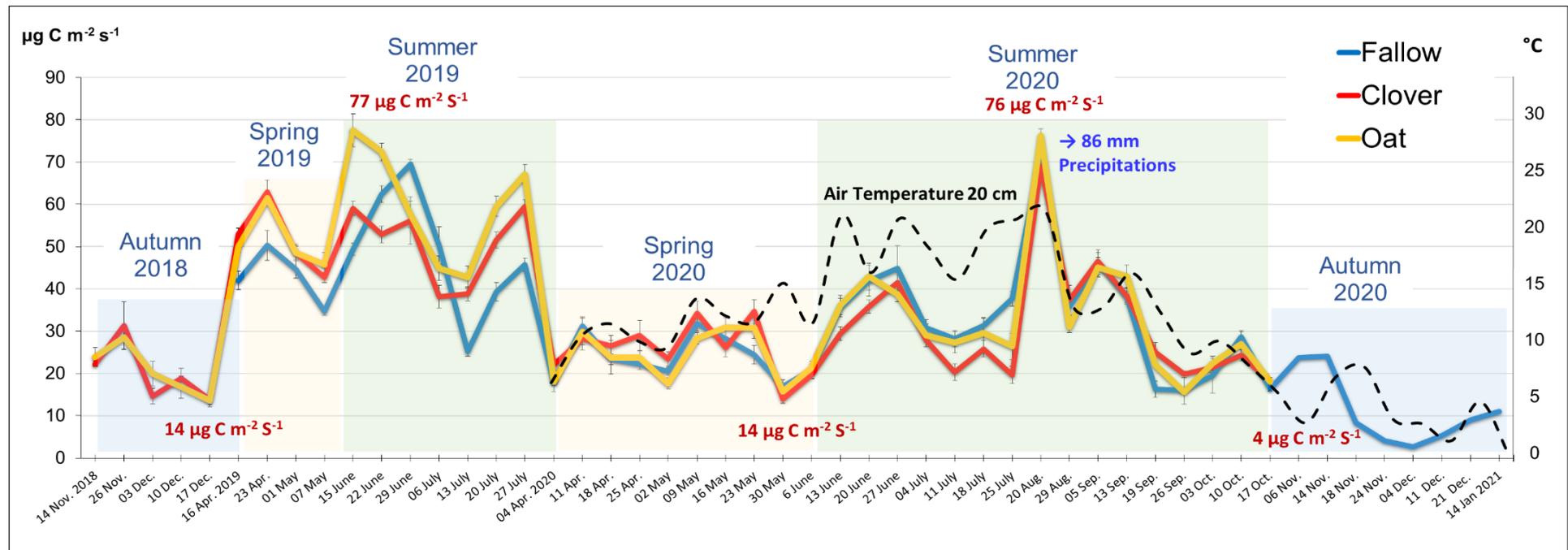


Figure 21: Dynamic of CO₂ efflux (Fc) rate ($\mu\text{g C m}^{-2} \text{s}^{-1}$) of the soil surface from Nov. 2018 till Jan. 2021, BNF – Justus Liebig University Giessen. The lines indicate three different land uses including clover mulch (red line), oat (yellow line) and fallow (blue line). The dashed black line represents the air temperature (only in 2020). In total 34 times of measurements were carried out; each time includes 9 hours measurement from morning to evening.

In the subsequent **growing season 2020**, the near surface Fc dynamic (min. – max.) was quite high over the seasons from spring to winter. In spring (April – May) the higher Fc level was observed by clover mulch (13.9 – 34.6 $\mu\text{g C m}^{-2} \text{s}^{-1}$) compared to oat (15.7 – 30.9 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and fallow (16.9 – 31.8 $\mu\text{g C m}^{-2} \text{s}^{-1}$) (table 43). In addition, the plots with optimal/maximal dosage of mineral fertilization (PK+100% N) reached the higher level (19.7 – 37.4 $\mu\text{g C m}^{-2} \text{s}^{-1}$) of Fc compared to No fertilization (17.4 – 28.7 $\mu\text{g C m}^{-2} \text{s}^{-1}$).

In summer 2020, oat (21.2 – 76.2 $\mu\text{g C m}^{-2} \text{s}^{-1}$) achieved steady higher Fc dynamic compared to clover (19.6 – 69.1 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and fallow (20.8 – 71.5 $\mu\text{g C m}^{-2} \text{s}^{-1}$). Furthermore, the Fc variation within the treatment of optimal dosage of NPK fertilization was pretty high (22.6 – 84.4 $\mu\text{g C m}^{-2} \text{s}^{-1}$) compared to No fertilization (18.6 – 60.2 $\mu\text{g C m}^{-2} \text{s}^{-1}$). In autumn (Sep. – Oct.), as expected, the Fc variation was decreased caused by decreasing air temperatures. But the maximum Fc efflux in autumn was still higher (45 – 47 $\mu\text{g C m}^{-2} \text{s}^{-1}$) in autumn than in spring (29 – 37 $\mu\text{g C m}^{-2} \text{s}^{-1}$). In autumn, the Fc variation was recorded at 15.6 to 47 $\mu\text{g C m}^{-2} \text{s}^{-1}$ among the treatments (table 43). In winter, as the air temperature dropped down, the lowest level (2.6 – 24.1 $\mu\text{g C m}^{-2} \text{s}^{-1}$) of near surface Fc was recorded.

Table 43: Effects of preceding crops (PC) and NPK fertilization (NPK) on CO₂ efflux (Fc) of the soil surface in the growing season 2020 (in winter rye from 04 Apr. to 17 Oct.) and in the winter period 2020 / 2021 (after plowing the soil from 06 Nov. to 14 Jan.), LTE “BNF” Giessen (Obs.: number of observations, SE: standard error).

CO₂ efflux (Fc) of the soil ($\mu\text{g C m}^{-2} \text{s}^{-1}$)						
Time period	Treatments	Min.	Max.	Mean	SE	Obs.
Spring 2020 (in winter rye)						
04 Apr. – 30 May	Fallow	16.9	31.8	24.1	1.9	9
04 Apr. – 30 May	Clover	13.9	34.6	26.4	2.3	9
04 Apr. – 30 May	Oat	15.7	30.9	24.3	1.9	9
04 Apr. – 30 May	No fertilization	17.4	28.7	22.8	2.0	9
04 Apr. – 30 May	PK+100% N	19.7	37.4	29.2	2.2	9
Summer 2020 (in winter rye)						
06 June – 30 Aug.	Fallow	20.8	71.5	37.9	2.4	10
06 June – 30 Aug.	Clover	19.6	69.1	32.6	2.0	10
06 June – 30 Aug.	Oat	21.2	76.2	35.9	2.3	10
06 June – 30 Aug.	No fertilization	18.6	60.2	32.6	2.3	10
06 June – 30 Aug.	PK+100% N	22.6	84.4	38.3	2.1	10
Autumn 2020 (after harvest of winter rye)						
05 Sep. – 17 Oct.	Fallow	16.0	46.3	25.9	1.9	7
05 Sep. – 17 Oct.	Clover	18.0	46.5	27.6	1.6	7
05 Sep. – 17 Oct.	Oat	15.6	45.1	27.6	1.6	7
05 Sep. – 17 Oct.	No fertilization	17.0	45.1	25.1	1.9	7
05 Sep. – 17 Oct.	PK+100% N	16.9	46.8	28.9	1.5	7
Winter 2020 / 2021 (after plowing)						
06 Nov. – 14 Jan.	No treatment	2.6	24.1	11.0	4.1	8

During the first year in 2019, significant effects were found caused by different land uses during the growing season. In contrary, during the second year no significant effects were found by different land uses/pre-crops, only in few measurements a mineral NPK effect was

observed (table 44). Through the campaign 2019, on 16th and 23rd of April clover mulch (52.9 and 63.0 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and oat (49.9 and 61.6 $\mu\text{g C m}^{-2} \text{s}^{-1}$) led to significantly increased CO₂ efflux of the topsoil compared to fallow (42.0 and 50.3 $\mu\text{g C m}^{-2} \text{s}^{-1}$), respectively. On 15th and 22nd of June, oat (77.5 $\mu\text{g C m}^{-2} \text{s}^{-1}$ and 72.5 $\mu\text{g C m}^{-2} \text{s}^{-1}$) effectively increased the CO₂ efflux of the soil surface compared to clover mulch (59.0 and 52.8 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and fallow (49.3 and 62.4 $\mu\text{g C m}^{-2} \text{s}^{-1}$). On 13th, 20th and 27th of July clover mulch (38.9, 51.6 and 59.5 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and oat (42.7, 59.5 and 67.1 $\mu\text{g C m}^{-2} \text{s}^{-1}$) significantly increased the topsoil CO₂ efflux rate compared to fallow (25.2, 39.2 and 45.7 $\mu\text{g C m}^{-2} \text{s}^{-1}$), respectively (table 44).

On the other hand, during the campaign 2020, on 9th May (37.4 $\mu\text{g C m}^{-2} \text{s}^{-1}$), 6th June (22.6 $\mu\text{g C m}^{-2} \text{s}^{-1}$), 25th July (36.0 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and 20th August (84.4 $\mu\text{g C m}^{-2} \text{s}^{-1}$), the optimal/maximal dosage of mineral NPK fertilization (PK+100% N) has significantly increased the near surface CO₂ efflux among the treatments compared to no fertilization (25.4, 18.6, 19.8 and 60.2 $\mu\text{g C m}^{-2} \text{s}^{-1}$), respectively (table 44).

Table 44: Effect of preceding crops (PC) and NPK fertilization (NPK) on CO₂ efflux (Fc) of the soil in the two vegetation periods in 2019 and 2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means.

Vegetation period 2019 – CO ₂ efflux (Fc) in $\mu\text{g C m}^{-2} \text{s}^{-1}$					
Date of measurement	Fallow	Clover mulch	Oat	p-value	LSD 5%
16 April	42.0 b	52.9 a	49.9 ab	0.042	10.4
23 April	50.3 b	63.0 a	61.6 a	0.017	11.0
15 June	49.3 b	59.0 b	77.5 a	0.002	12.0
22 June	62.4 b	52.8 c	72.5 a	< 0.001	8.12
13 July	25.2 b	38.9 a	42.7 a	0.010	13.3
20 July	39.2 b	51.6 a	59.5 a	0.001	11.5
27 July	45.7 b	59.5 a	67.1 a	0.001	12.1
Vegetation period 2020 – CO ₂ efflux (Fc) in $\mu\text{g C m}^{-2} \text{s}^{-1}$					
Date of measurement	No fertilization	PK+100% N	p-value	LSD 5%	
9 May	25.4 b	37.4 a	0.017	11.6	
6 June	18.6 b	22.6 a	0.050	3.95	
25 July	19.8 b	36.0 a	0.018	15.8	
20 August	60.2 b	84.4 a	0.005	19.1	

In the year 2020 the near surface CO₂ efflux was subject to a strong fluctuation in summer (June - August) (38.5 $\mu\text{g C m}^{-2} \text{s}^{-1}$) compared to spring (April - May) (24.9 $\mu\text{g C m}^{-2} \text{s}^{-1}$) and autumn (September – October) (26.4 $\mu\text{g C m}^{-2} \text{s}^{-1}$), accordingly the CO₂ efflux declined in winter (November – December) (10.3 $\mu\text{g C m}^{-2} \text{s}^{-1}$) caused by frost (figure 22 and 23).

In the year 2019, the spring (April - May) and summer (June - July) rate of CO₂ efflux was recorded at 48.7 and 60.2 $\mu\text{g C m}^{-2} \text{s}^{-1}$, respectively (data not shown).

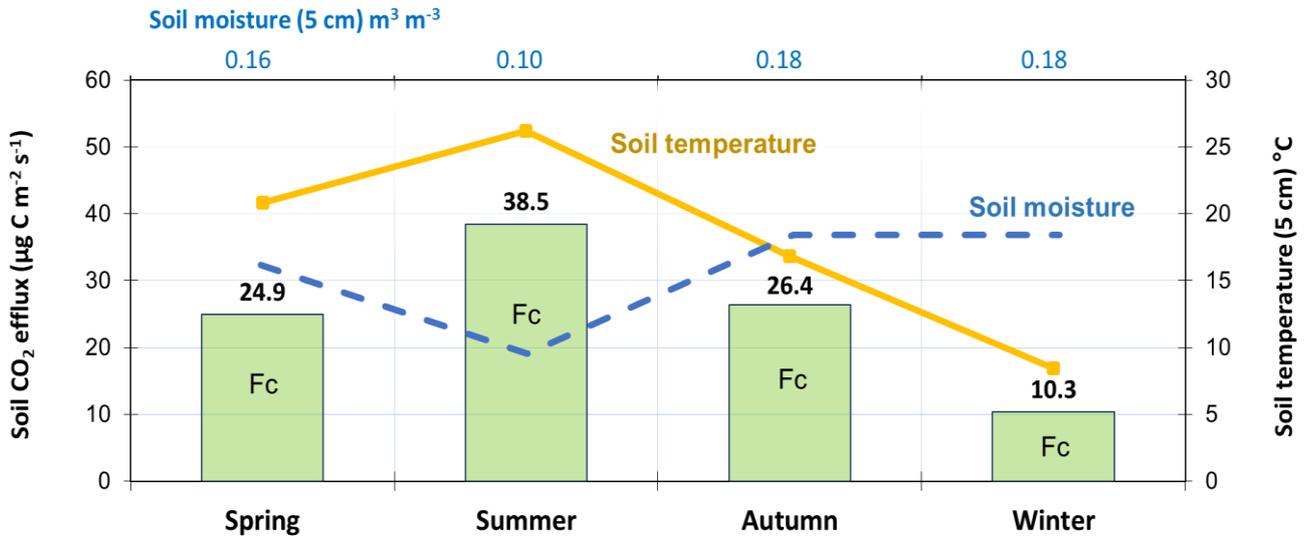


Figure 22: Seasonal variation of soil CO₂ efflux (F_c) in relation to soil temperature and soil moisture during the growth of winter rye in 2020, LTE “BNF”, experimental station Weilburger Grenze, JLU Giessen.

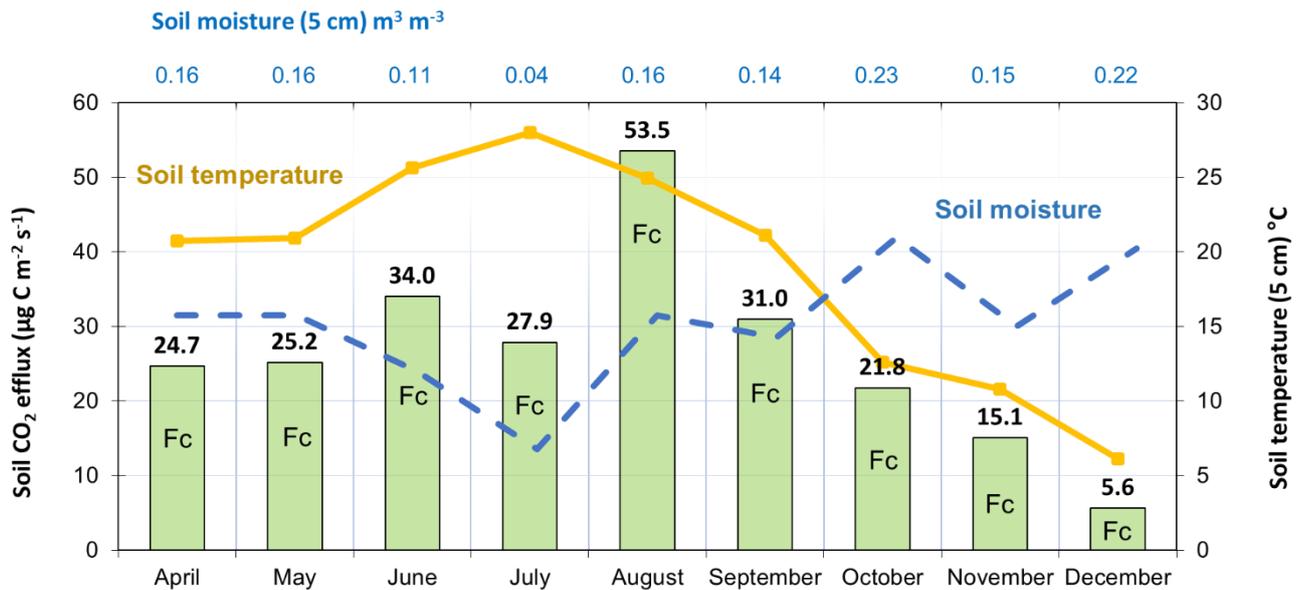


Figure 23: Monthly variation of soil surface CO₂ efflux (F_c) in relation to soil temperature and soil moisture during the season 2020, LTE “BNF”, experimental station Weilburger Grenze, JLU Giessen.

C efflux per day and season

In the first campaign, the results are derived from sixteen observations during the growing season 2018/19 from 14th of November to 27th of July, which included 1920 pairs of measurements. In 2018/19, the first year after cultivation of preceding crop C efflux of 3.6 g C m⁻² day⁻¹ resulting in 109 g C month⁻¹ and in total of 1321 g C year⁻¹ (table 45).

In contrary, the second campaign with thirty-four observations and 4080 pairs measurements during the growing season 2020/21 from 4th of April to 14th of January presented lower C effluxes compared to the first campaign. Thus, the C efflux was 2.2 g C m⁻² day⁻¹ resulting in 65 g C month⁻¹ and in total 787 g C year⁻¹ (table 45).

Table 45: C efflux of the soil surface per day, month and year in the LTE “BNF” Giessen, measurement campaigns 2018/2019 and 2020/2021 (Obs. = number of observations).

	Campaign 14. 11. 2018 to 27. 07. 2019				Campaign 04. 04. 2020 to 14. 01. 2021			
	µg C m ⁻²	g C m ⁻²			µg C m ⁻²	g C m ⁻²		
	s ⁻¹	day ⁻¹	month ⁻¹	year ⁻¹	s ⁻¹	day ⁻¹	month ⁻¹	year ⁻¹
Min.	13.7	1.2	35	431	2.6	0.2	7	83
Max.	62.5	5.4	162	1972	72.4	6.3	188	2282
Mean	41.9	3.6	109	1321	25.0	2.2	65	787
Obs.	16	16	16	16	34	34	34	34

Based on the measurements made it can be stated that the C efflux of the soil surface varied between the seasons. Our measurement includes three seasons (winter 2018, spring 2019 and summer 2019) from 14 November 2018 to 27 July 2019. In winter (14 November – 17 December), a lower Fc efflux of 1.77 g CO₂-C m⁻² day⁻¹ over five observations were observed and the variation ranged from minimum 1.18 to maximum 2.59 g CO₂-C m⁻² day⁻¹. Whereas, in the spring (16 April – 7 May) the Fc level of the soil surface within four observations increased to 4.25 g CO₂-C m⁻² day⁻¹ (with variation of 3.55 to 5.04 g CO₂-C m⁻² day⁻¹). In summer (15 June – 27 July), we observed a further increasing of Fc to the highest level of 5.18 g CO₂-C m⁻² day⁻¹ by seven observations ranging from 3.08 to 5.40 g CO₂-C m⁻² day⁻¹ (table 46).

In the second year the same tendency but with lower values was observed. The campaign 2020/21, which includes four seasons (spring, summer, autumn and winter), included thirty-four observations from 4 April 2020 to 14 January 2021. In spring (4 April – 30 May), the Fc level of the soil surface within nine observations recorded to 2.16 g CO₂-C m⁻² day⁻¹ (varying from 1.34 to 2.72 g CO₂-C m⁻² day⁻¹). In summer (6 June – 29 August) within ten observations the Fc value increases to 3.06 g CO₂-C m⁻² day⁻¹ (with variation of 1.77 to 6.24 g CO₂-C m⁻² day⁻¹). In autumn (5 September – 17 October), the Fc value of 2.33 g CO₂-C m⁻² day⁻¹ was observed within seven measurements, and the variation was 1.48 to 3.97 g CO₂-C m⁻² day⁻¹. In winter (6 November – 14 January), within eight observations the Fc value decreased to the lowest level of 0.88 g CO₂-C m⁻² day⁻¹ (with variation of 0.23 to 2.08 g CO₂-C m⁻² day⁻¹) (table 46).

Table 46: Seasonal C/CO₂ efflux of the soil surface in the LTE “BNF” experimental station Giessen 2018-2021.

	Campaign 14 11 2018 to 27 07 2019			Campaign 04 04 2020 to 14 01 2021			
	g CO ₂ -C m ⁻² day ⁻¹			g CO ₂ -C m ⁻² day ⁻¹			
	Winter	Spring	Summer	Spring	Summer	Autumn	Winter
Min.	1.18	3.55	3.08	1.34	1.77	1.48	0.23
Max.	2.59	5.04	5.40	2.72	6.24	3.97	2.08
Mean	1.77	4.25	5.18	2.16	3.06	2.33	0.88
Obs.	5	4	7	9	10	7	8

Correlations of CO₂ efflux with air/soil temperature and soil moisture

Based on the data from November 2018 to July 2019 a strong positive correlation was found between CO₂ efflux on one hand and air temperature ($r = 0.85$) and temperature soil ($r = 0.80$) on the other hand (table 47). In the year 2019, soil moisture measurement was done from 15th of June to 27th of July with Stevens probe and no clear correlations was observed between soil CO₂ efflux measurement and the soil moisture ($r = -0.26$). The temperature inside the chamber was also positively responded to the soil emission ($r = 0.90$).

Table 47: Correlation matrix for the relationships between Fc and meteorological parameters, LTE-BNF 2019, ns: not significant.

Parameters	Fc 1	Fc 2	CO ₂	SM	ST 5 cm	AT 2 m	CT 5 cm	AT 20 cm
	μmol m ⁻² s ⁻¹	μg m ⁻² s ⁻¹	ppmv	m ³ m ⁻³	°C	°C	°C	°C
Fc 1 ¹	1							
Fc 2 ²	1	1						
CO ₂ ³	0.28 ^{ns}	0.28 ^{ns}	1					
SM ⁴	-0.26 ^{ns}	-0.26 ^{ns}	0.14 ^{ns}	1				
ST 5 cm ⁵	0.80 ^{**}	0.80 ^{**}	0.38 ^{ns}	-0.66 ^{ns}	1			
AT 2 m ⁶	0.84 ^{***}	0.84 ^{***}	0.49 ^{ns}	-0.66 ^{ns}	0.96 ^{***}	1		
CT 5 cm ⁷	0.90 ^{***}	0.90 ^{***}	0.30 ^{ns}	-0.65 ^{ns}	0.93 ^{***}	0.96 ^{***}	1	
AT 20 cm ⁶	0.85 ^{***}	0.85 ^{***}	0.49 ^{ns}	-0.69 ^{ns}	0.97 ^{***}	1.00 ^{***}	0.96 ^{***}	1

¹ Fc 1: Flux rate in μmol m⁻² s⁻¹, ² Fc 2: Flux rate in μg m⁻² s⁻¹, ³ CO₂: CO₂ concentration, ⁴ SM: Soil Moisture, ⁵ ST: Soil Temperature, ⁶ AT: Air Temperature, ⁷ CT: Air temperature within the chamber.

The results of the regression analysis investigating the relationship between CO₂ efflux of the soil (Fc) and the environmental/meteorological parameters within the campaign from autumn 2018 to summer 2019 are presented in table 48. It was found that the efflux rate (Fc) was positively correlated with the soil temperature (ST 5 cm) represented by $r = 0.80$ ($p = 0.001$). Furthermore, a positive relationship was also determined between Fc on the one hand and air temperature at 2 m (AT 2m, $r = 0.84$), air temperature within the chamber (CT 5 cm, $r = 0.90$) and air temperature at 20 cm (AT 20 cm on the other hand. However, no significant relationship was found between Fc and the soil moisture (5 cm) (table 48).

Table 48: Correlation coefficients (r) based on linear regression for the relationship between CO₂ efflux (Fc) and CO₂ concentration of the air, air/soil temperature and soil moisture, BNF Giessen, 2018-2019; ns: not significant.

Parameters	CO ₂ ¹	SM ²	ST 5 cm ³	AT 2 m ⁴	CT 5 cm ⁵	AT 20 cm ⁴
	ppmv	m ³ m ⁻³	°C	°C	°C	°C
r	0.28	0.26	0.80	0.84	0.90	0.85
p -value	0.29	0.57	0.001	< 0.001	< 0.001	< 0.001
	ns	ns	***	***	***	***

¹ CO₂: CO₂ concentration, ² SM: Soil Moisture, ³ ST: Soil Temperature, ⁴ AT: Air Temperature, ⁵ CT: Air temperature within the chamber.

In the year 2020, the same relationship was found compared to the year before. Thus, a positive correlation was observed between CO₂ flux rate and air temperature ($r = 0.75$) and between Fc and soil temperature ($r = 0.75$) (table 49). In addition, the chamber temperature was also positive correlated with CO₂ efflux ($r = 0.73$). In this year no clear correlation was observed between soil CO₂ efflux data and the soil moisture ($r = -0.21$).

Table 49: Correlation matrix for the relationships between Fc and meteorological parameters, LTE BNF Giessen 2020.

Parameters	Fc 1	Fc 2	CO ₂	SM	ST 5 cm	AT 2 m	CT 5 cm	AT 20 cm
	μmol m ⁻² s ⁻¹	μg m ⁻² s ⁻¹	ppmv	m ³ m ⁻³	°C	°C	°C	°C
Fc 1 ¹	1							
Fc 2 ²	1	1						
CO ₂ ³	-0.33 ^{ns}	-0.33 ^{ns}	1					
SM ⁴	-0.21 ^{ns}	-0.21 ^{ns}	0.41 [*]	1				
ST 5 cm ⁵	0.72 ^{***}	0.72 ^{***}	-0.64 ^{***}	-0.65 ^{***}	1			
AT 2 m ⁶	0.76 ^{***}	0.76 ^{***}	-0.61 ^{***}	-0.58 ^{***}	0.94 ^{***}	1		
CT 5 cm ⁷	0.73 ^{***}	0.73 ^{***}	-0.64 ^{***}	-0.64 ^{***}	1.00 ^{***}	0.95 ^{***}	1	
AT 20 cm ⁶	0.75 ^{***}	0.75 ^{***}	-0.64 ^{***}	-0.59 ^{***}	0.95 ^{***}	1.00 ^{***}	0.96 ^{***}	1

¹ Fc 1: Flux rate in μmol m⁻² s⁻¹, ² Fc 2: Flux rate in μg m⁻² s⁻¹, ³ CO₂: CO₂ concentration, ⁴ SM: Soil Moisture, ⁵ ST: Soil Temperature, ⁶ AT: Air Temperature, ⁷ CT: Air temperature within the chamber of the device.

The result of regression analysis of the second campaign from spring 2020 to winter 2020 revealed that the efflux rate (Fc) was positively correlated with soil temperature (ST 5 cm) represented by $r = 0.72$, ($p = 0.001$) (table 50). Furthermore, a positive relationship was also determined between Fc and the air temperature at 2 m ($r = 0.76$), air temperature at 20 cm ($r = 0.75$) as well as chamber temperature (CT) at 5 cm ($r = 0.73$). However, no clear relationship was found between Fc and the soil moisture in 5 cm ($r = 0.21$) and only a weak correlation was observed with CO₂ concentration (table 50).

Table 50: Correlation coefficients (r) based on linear regression for the relationship between CO₂ efflux (Fc) and CO₂ concentration of the air, air/soil temperature and soil moisture, BNF Giessen, 2020-2021; ns: not significant.

Parameters	CO ₂ ¹	SM ²	ST 5 cm ³	AT 2 m ⁴	CT 5 cm ⁵	AT 20 cm ⁴
	ppmv	m ³ m ⁻³	°C	°C	°C	°C
r	0.33	0.21	0.72	0.76	0.73	0.75
p -value	0.06	0.24	< 0.001	< 0.001	< 0.001	< 0.001
	ns	ns	***	***	***	***

¹ CO₂: CO₂ concentration, ² SM: Soil Moisture, ³ ST: Soil Temperature, ⁴ AT: Air Temperature, ⁵ CT: Air temperature within the chamber.

4. 2 Effect of preceding crops and mineral NPK fertilization on plant parameters

4. 2. 1 Number of vegetative tillers and spike density

The formation of vegetative tillers is a botanical characteristic of all cereal species. They are a prerequisite for the formation of spikes and thus represent the most important yield component of all cereal crop species.

The number of vegetative tillers was determined in 2016 and 2017. The ANOVA evaluation showed that crimson clover led to significant higher tiller density in winter rye in 2016 (741 tillers m⁻²) (figure 24). Surprisingly, field bean had statistically the same tiller density as fallow (619 tillers m⁻²), oat (609 tillers m⁻²) and maize (569 tillers m⁻²). In 2017, in summer barley, no significant effects by pre-crops were found, while crimson clover had still the highest number of tillers per square meter (971 tillers m⁻²).

Further on, in 2016, in winter rye there was an interaction between pre-crops and NPK fertilization contrary to 2017 (figure 24). This interaction shows that crimson clover significantly increased the positive effect of PK+100% N compared to PK+50% N, while after maize there was no difference between 50% and 100% N (figure 24).

Furthermore, in both years (2016 and 2017) there was a significant effect by PK+100% N application (table 51). So that in 2016 the higher tiller density (846 tillers m⁻²) was achieved by optimal dosage of NPK fertilization. In contrary, in 2017 the higher number of tillers was observed by the optimal dosage of NPK fertilization (995 tillers m⁻²) and PK 50% (946 tillers m⁻²) respectively.

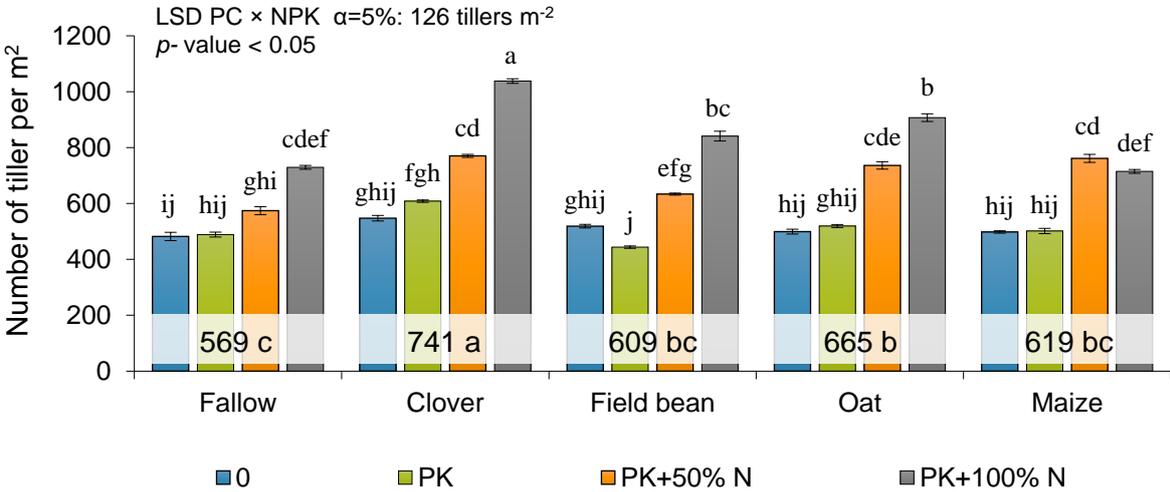


Figure 24: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on number of tillers in winter rye in 2016, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Table 51: Effect of pre-crops (PC) and mineral fertilization (NPK) on number of tillers in winter rye 2016 and summer barley 2017, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment; ns: not significant.

Treatments		Number of tillers (tillers m ⁻²)	
		2016	2017
Preceding crop (PC)	Fallow	569 c	920 ns
	Crimson clover	741 a	971 ns
	Field bean	609 bc	916 ns
	Oat	665 b	868 ns
	Maize	619 bc	934 ns
NPK fertilization (NPK)	No fertilization	509 c	858 b
	PK 50%	512 c	946 ab
	PK+50% N	695 b	888 b
	PK+100% N	846 a	995 a
p-value	PC	< 0.001	0.403
	NPK	< 0.001	0.024
	PCxNPK	0.035	0.487
LSD 5% (tillers m ⁻²)	PC	63	104
	NPK	56	93
	PCxNPK	126	209

Further on it was observed that crimson clover as a green mulch and the full dosage of mineral fertilization (PK+100% N) has improved the spike density along the crop rotation in 2015 to 2020 (table 52). So that in 2015, in winter wheat, as the first crop after the preceding crops/land uses, the highest spikes number of 558 spikes m⁻² after crimson clover compared to field bean (470 spikes m⁻²) and fallow land (461 spikes m⁻²) was found (table 52). Moreover, oat with 382 spikes m⁻² and maize with 425 spikes m⁻² reached the lowest values.

In contrary, in 2016 no significant effects of pre-crops neither by legumes nor cereals were found. There was only a tendency of higher values after crimson clover and field bean. In addition, in this year, the spike density of winter rye had a positive response to mineral N fertilization (p-value < 0.001). Full dosage of N fertilization (PK+100% N) increased spike density at the levels of 591 spikes m⁻² (table 52).

In 2017, in the fourth crop rotation in summer barley, the same tendency as 2016 was found. Spike density only responded to mineral N fertilization at both levels. Therefore, by applying nitrogen fertilization the spike density increased to 708 spikes m⁻² (PK+ 50% N) and 808 spikes m⁻² (PK+100% N) respectively (table 52).

Table 52: Effect of preceding crop (PC) and NPK fertilization (NPK) on spike density 2015-2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment; ns: not significant.

Treatments		Spike densities (spikes m ⁻²)				
		2015	2016	2017	2019	2020
Preceding crop (PC)	Fallow	461 b	410 ns	655 ns	512 ab	326 b
	Crimson clover	558 a	459 ns	690 ns	570 a	400 a
	Field bean	470 b	458 ns	686 ns	494 abc	311 b
	Oat	382 c	421 ns	681 ns	429 c	365 ab
	Maize	425 bc	425 ns	764 ns	480 bc	368 ab
NPK fertilization (NPK)	No fertilization	384 c	327 c	603 c	448 bc	282 c
	PK 50%	409 c	329 c	661 bc	413 c	277 c
	PK+50% N	488 b	492 b	708 b	500 b	364 b
	PK+100% N	556 a	591 a	808 a	628 a	493 a
p-value	PC	< 0.001	0.190	0.091	< 0.001	0.036
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.790	0.985	0.084	0.490	0.649
LSD 5% (spikes m ⁻²)	PC	54	24	80	80	60
	NPK	48	45	71	67	54
	PCxNPK	107	101	159	114	121

In 2019, the same tendency as in the years before was found. So that crimson clover (with 570 spikes m⁻²) and field bean (with 494 spikes m⁻²) reached the highest spike density compared to fallow land (as a kind of control) (table 52). In this year oat has had the lowest level with 429 spikes m⁻². The optimal dosage of mineral fertilization improved the spike density (PK+100% N with 628 spikes m⁻²). Although, in 2020 in winter rye, crimson clover reached the highest spikes number (400 spikes m⁻²), this was statistically identical with that of oat (365 spikes m⁻²) and maize (368 spikes m⁻²) reaching slightly lower values.

4. 2. 2 Plant height

According to statistical analysis there was a significant effect of legumes/cereals used as pre-crop and mineral N fertilization in both levels on plant height of subsequent crops during the rotational crop sequence in 2015-2020. In 2016 was observed that the plant height of winter rye which was grown in the third year of the rotation was at the highest level after crimson clover (101 cm) but unfortunately, no clear effect of previous crop on plant height (p-value = 0.317) was found. In contrary, N fertilization has significantly increased the plant height of rye. So that, PK+100% N increased the plant height to the maximum level (109 cm) compared to PK 50% and fallow land (93 cm).

In 2017 in summer barley, the last year of the crop rotation (fourth year) the same tendency was found after crimson clover (72 cm) and field bean (72 cm) where the plant height was at the heighest level. Similar result was found in 2019 in winter wheat when crimson clover (81 cm) and PK+100% N (83 cm) was reached the maximum plant height respectively. In this year, surprisingly field bean (72 cm) increased the plant height identical with fallow land (72

cm) and maize (71 cm) respectively (table 53). Also in 2020, the height of winter rye was increased only by mineral N fertilization on the same levels of 96 cm (PK+50% N) and 97 cm (PK+100% N).

Table 53: Effect of preceding crop and NPK fertilization on plant height 2016-2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment; ns: not significant.

Treatments		Plant height (cm)			
		2016	2017	2019	2020
Preceding crop (PC)	Fallow	98 ns	66 c	72 b	93 ns
	Crimson clover	101 ns	72 a	81 a	93 ns
	Field bean	99 ns	72 ab	72 b	92 ns
	Oat	100 ns	68 bc	66 c	93 ns
	Maize	98 ns	69 abc	71 b	92 ns
NPK fertilization (NPK)	No fertilization	93 c	63 c	65 c	90 b
	PK 50%	93 c	62 c	65 c	89 b
	PK+50% N	102 b	72 b	76 b	96 a
	PK+100% N	109 a	79 a	83 a	97 a
p-value	PC	0.317	0.011	< 0.001	0.818
	NPK	< 0.001	< 0.001	< 0.001	< 0.001
	PC×NPK	0.920	0.953	0.352	0.958
LSD 5% (cm)	PC	4	4	4	4
	NPK	3	4	4	3
	PC×NPK	8	8	6	7

4. 2. 3 Leaf Area Index (LAI)

Based on statistical analysis LAI was positively and significantly influenced by preceding crops and mineral fertilization during the whole trial period from 2015 to 2020 (table 54). So that in the second year of the rotation in winter wheat 2015 crimson clover as a green mulch led to increased LAI values to the highest levels of 3.3 (29 May), 3.8 (5 June) and 3.3 (16 June) compared to the fallow land.

Furthermore, additional mineral N fertilization (PK+100% N) improved the LAI values through the growing season as follows: 3.7 (29 May), 4.2 (5 June) and 3.7 (16 June) compared to the control (no fertilization) 1.7 (table 54). In addition to this, in the third rotational year in winter rye 2016 the increase of LAI was influenced by the interaction between both test factors previous crop and NPK fertilization (figure 25). This shows that mineral N fertilization was more effective on LAI of winter rye at DC 73 after clover mulch then after fallow land.

Table 54: Effect of preceding crop and NPK fertilization on LAI 2015-2016, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment; ns: not significant.

Treatments		Leaf Area Index (LAI)						
		2015			2016			
		29 May	05 June	16 June	12 April	05 May	12 May	01 June
Preceding Crop (PC)	Fallow	2.50 c	2.69 c	2.39 c	1.04 c	1.56 d	1.93 b	2.03 c
	Crimson clover	3.33 a	3.84 a	3.31 a	1.53 a	2.17 a	2.64 a	2.76 a
	Field bean	2.81 b	3.13 b	2.94 b	1.44 ab	2.00 b	2.39 a	2.47 b
	Oat	1.92 d	2.13 d	1.97 d	1.36 ab	1.93 bc	2.30 a	2.35 b
	Maize	2.11 d	2.26 d	2.16 cd	1.23 bc	1.77 cd	2.36 a	2.34 b
NPK fertilization (NPK)	No fertilization	1.74 c	1.91 c	1.79 c	0.88 c	1.14 c	1.37 c	1.40 c
	PK 50%	1.74 c	1.87 c	1.70 c	0.92 c	1.21 c	1.36 c	1.42 c
	PK+50% N	2.98 b	3.26 b	2.97 b	1.52 b	2.16 b	2.62 b	2.71 b
	PK+100% N	3.68 a	4.20 a	3.75 a	1.96 a	3.04 a	3.96 a	4.05 a
p-value	PC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PC×NPK	0.734	0.991	0.464	0.864	0.150	0.379	0.004
LSD 5%	PC	0.27	0.32	0.28	0.19	0.14	0.27	0.15
	NPK	0.24	0.28	0.25	0.17	0.13	0.24	0.14
	PC×NPK	0.54	0.63	0.56	0.39	0.28	0.54	0.31

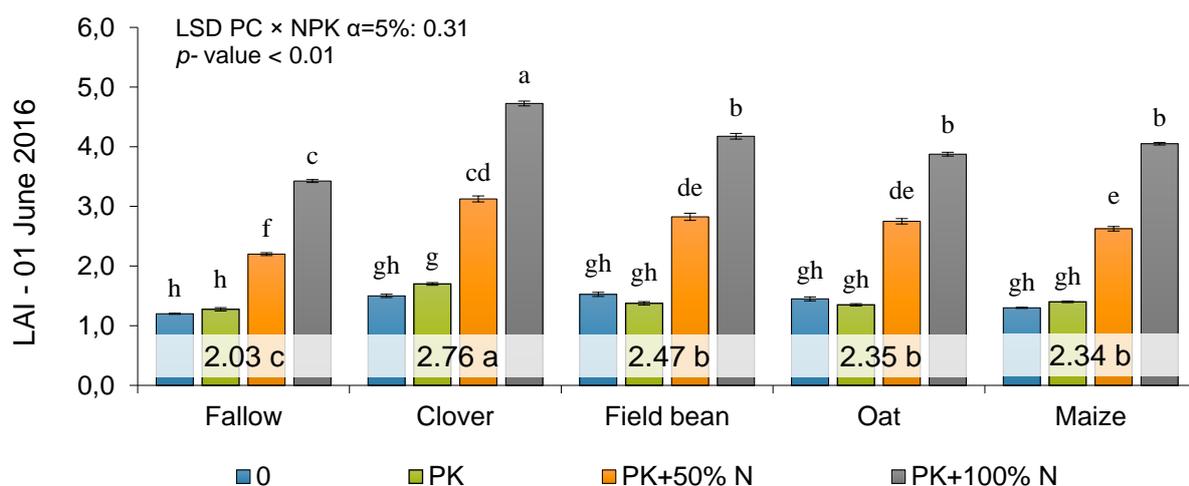


Figure 25: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on LAI of winter rye at DC 73 (grain fill period) in June 2016, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

In addition, in winter wheat 2019 (two years after the preceding crops) legumes were better than cereals indicated by increased LAI values of about 4.5 and 5 at second node stage DC 32 and heading stage DC 50 respectively (figure 26). Also, mineral N at both levels (PK+50% N and PK+100% N) has increased the LAI values during the growing season (figure 27). In contrary, in winter rye 2020, crimson clover significantly influenced the LAI at milk development stage (DC 73) but the positive effect of mineral N fertilization was plausible (table 55).

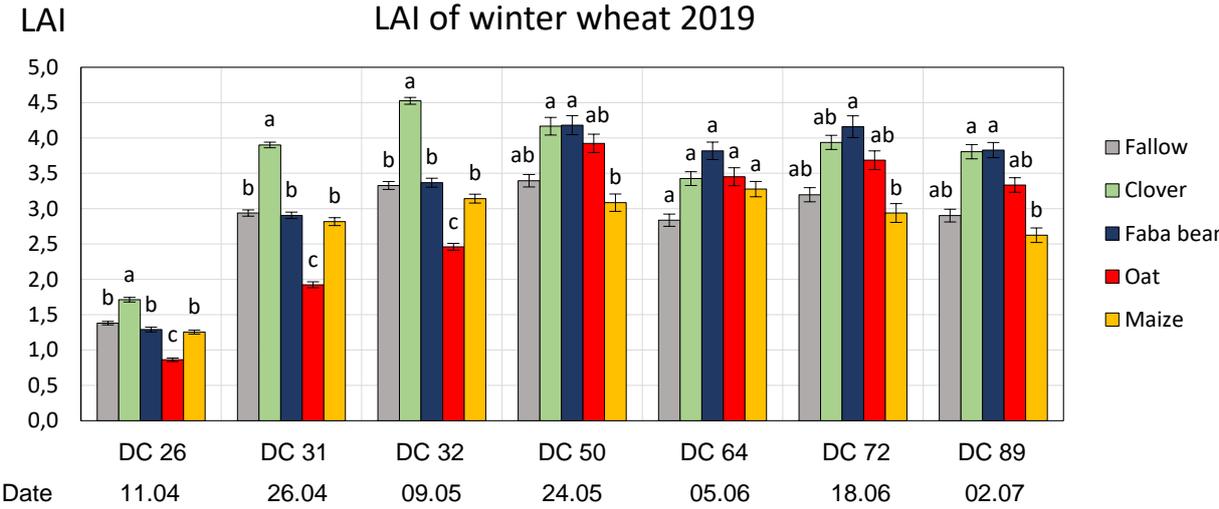


Figure 26: Effect of pre-crops (PC) on LAI in winter wheat 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

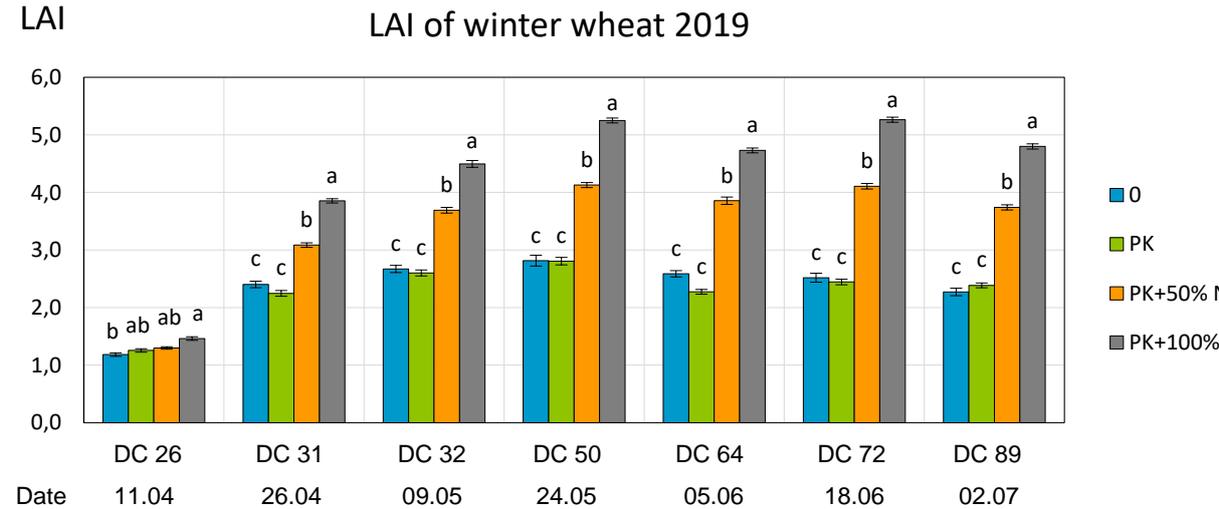


Figure 27: Effect of mineral fertilization (NPK) on LAI in winter wheat 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Table 55: Effect of preceding crops and NPK fertilization on LAI of winter rye 2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment; ns: not significant.

Treatments		Leaf Area Index (LAI)			
		2020			
		29 April (DC 34)	14 May (DC 51)	29 May (DC 65)	14 June (DC 73)
Preceding crop (PC)	Fallow	0.62 ns	0.74 ns	0.70 ns	1.26 b
	Crimson clover	0.69 ns	0.81 ns	0.76 ns	1.46 a
	Field bean	0.58 ns	0.83 ns	0.72 ns	1.12 b
	Oat	0.62 ns	0.90 ns	0.68 ns	1.22 b
	Maize	0.59 ns	0.75 ns	0.59 ns	1.20 b
NPK fertilization (NPK)	No fertilization	0.49 c	0.51 c	0.47 c	0.83 c
	PK 50%	0.48 c	0.55 c	0.37 c	0.75 c
	PK+50% N	0.68 b	0.97 b	0.84 b	1.53 b
	PK+100% N	0.83 a	1.21 a	1.07 a	1.90 a
p-value	PC	0.413	0.276	0.130	< 0.001
	NPK	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.484	0.217	0.623	0.741
LSD 5%	PC	0.12	0.16	0.13	0.18
	NPK	0.11	0.14	0.12	0.16
	PCxNPK	0.24	0.31	0.26	0.36

4. 2. 4 Vegetation Indices (NDVI/NDRE)

To evaluate the plant growth of cereals crops the vegetative indices NDVI and NDRE were measured every two weeks during the vegetative periods from 2016 to 2020. Based on the analysed data there was a positive relationship between NDVI and NDRE values. Both parameters were significantly increased during the vegetative period by legumes used as previous crops as well as mineral nitrogen fertilization at both levels (PK+50% N and PK+100% N). In the year 2016, in winter rye, the third year of the rotation, NDVI value at first node development stage DC 31 was at the highest level (0.53) when crimson clover was used as a green manure in the rotation. In this year oat has recorded the same values during the growing season as crimson clover (table 56). In addition, mineral N fertilization in both levels (PK+50% N and PK+100% N) consecutively improved the NDVI values among plant development stages. At the stage DC 31 the additive N fertilization has increased the NDVI value from 0.54 to 0.62 when the optimal dosage of N (PK+100% N) was applied (table 56).

Regarding NDRE values, in the same year (2016), significant effects caused by preceding crops as well as NPK fertilization were found. Therefore, crimson clover used as a green mulch has increased the NDRE values to the maximum level of 0.45 at the second node development stage DC 32. While field bean has identical NDRE value (0.42) as maize (0.42). However, all

previous crops led to increase the NDRE values compared to fallow (0.38). Furthermore, mineral N fertilization at both levels improved the NDRE values. So that, by applying PK+100% N the NDRE value increased significantly to 0.59 and PK+50% N led to lower level of NDRE to 0.47 (table A 2). Accordingly, fallow led to the lowest NDRE value of 0.30 as it was expected.

Table 56: Effect of preceding crops and NPK fertilization on NDVI of winter rye 2016, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		Vegetation Index (NDVI)					
		2016					
		DC 31	DC 32	DC 34	DC 60	DC 75	DC 82
Preceding crop (PC)	Fallow	0.48 b	0.38 c	0.44 b	0.43 b	0.42 b	0.43 ns
	Crimson clover	0.53 a	0.46 a	0.53 a	0.49 a	0.49 a	0.46 ns
	Field bean	0.48 b	0.42 b	0.49 ab	0.47 ab	0.46 ab	0.44 ns
	Oat	0.51 a	0.43 b	0.50 a	0.50 a	0.48 a	0.44 ns
	Maize	0.48 b	0.41 bc	0.48 ab	0.47 ab	0.46 ab	0.45 ns
NPK fertilization (NPK)	No fertilization	0.40 c	0.31 c	0.31 c	0.30 c	0.32 c	0.33 c
	PK 50%	0.42 c	0.32 c	0.31 c	0.29 c	0.35 c	0.34 c
	PK+50% N	0.54 b	0.46 b	0.60 b	0.61 b	0.56 b	0.51 b
	PK+100% N	0.62 a	0.59 a	0.74 a	0.70 a	0.62 a	0.60 a
p-value	PC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.601
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PC×NPK	0.25	0.43	0.48	0.10	0.28	0.86
LSD 5%	PC	0.02	0.03	0.05	0.04	0.04	0.04
	NPK	0.02	0.02	0.04	0.04	0.03	0.04
	PC×NPK	0.05	0.05	0.09	0.09	0.07	0.08

The statistical analysis of NDVI and NDRE in 2017 showed, in several plant development stages e.g. stem elongation (DC 32 and DC 37), heading stage (DC 51, DC 56 and DC 65) and grain development stage (DC 75) the vegetation indices were significantly increased (p value < 0.01) by crimson clover as well as by mineral N fertilization (table 57 and A 3). Accordingly, in summer barley 2017, the maximum levels of NDVI (0.71) and NDRE (0.25) were recorded at the development stage DC 32. In addition, additive mineral N has improved the NDVI value from 0.68 (PK+50% N) to 0.73 (PK+100% N) respectively (table 57). The same tendency is recorded regarding NDRE value as it was increased from 0.24 to 0.28 by duplication the N amount from PK+50% N to PK+100% N (table A 3).

Table 57: Effect of pre-crops and NPK fertilization on NDVI of summer barley 2017, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		Vegetation Index (NDVI)					
		2017					
		DC 32	DC 37	DC 51	DC 56	DC 65	DC 75
Preceding crop (PC)	Fallow	0.58 c	0.56 c	0.54 b	0.44 d	0.41 b	0.44 b
	Crimson clover	0.71 a	0.68 a	0.64 a	0.58 ab	0.50 a	0.52 a
	Field bean	0.68 ab	0.64 ab	0.64 a	0.54 ab	0.48 a	0.49 ab
	Oat	0.65 b	0.61 bc	0.58 b	0.51 cd	0.44 ab	0.51 a
	Maize	0.66 ab	0.61 bc	0.59 ab	0.48 cd	0.45 ab	0.49 ab
NPK fertilization (NPK)	No fertilization	0.62 b	0.55 c	0.53 c	0.41 c	0.36 c	0.42 c
	PK 50%	0.59 b	0.54 c	0.50 c	0.41 c	0.34 c	0.39 c
	PK+50% N	0.68 a	0.66 b	0.63 b	0.54 b	0.49 b	0.53 b
	PK+100% N	0.73 a	0.73 a	0.74 a	0.70 a	0.64 a	0.63 a
p-value	PC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.529	0.714	0.989	0.706	0.881	0.886
LSD 5%	PC	0.04	0.05	0.05	0.05	0.07	0.06
	NPK	0.04	0.04	0.04	0.04	0.06	0.05
	PCxNPK	0.08	0.09	0.09	0.09	0.10	0.09

In the 10th crop rotation similar NDVI/NDRE results were obtained as in the 9th crop rotation. Crimson clover and mineral N fertilization was significantly improved the vegetation indices in winter wheat 2019 and winter rye 2020 (table 58 and 59). Thus, in 2019 crimson clover improved the NDVI of winter wheat to the levels of 0.79 at second node stage. While field bean had, the same value as maize and fallow (table 58). Also, by duplicating mineral N from 90 kg ha⁻¹ to 180 kg ha⁻¹ the NDVI value was significantly increased to 0.82 / 0.83 at the stages DC 32 and DC 50, respectively.

Table 58: Effect of pre-crops and NPK fertilization on NDVI of winter wheat 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		Vegetation Index (NDVI) winter wheat						
		2019						
		DC 26	DC 31	DC 32	DC 50	DC 64	DC 72	DC 89
Preceding Crop (PC)	Fallow	0.58 b	0.68 b	0.70 b	0.65 b	0.67 b	0.52 b	0.22 b
	Crimson clover	0.66 a	0.77 a	0.79 a	0.78 a	0.77 a	0.61 a	0.29 ab
	Field bean	0.58 b	0.69 b	0.71 b	0.67 b	0.67 b	0.61 a	0.33 a
	Oat	0.46 c	0.58 c	0.60 c	0.56 c	0.57 c	0.59 a	0.28 ab
	Maize	0.58 b	0.69 b	0.69 b	0.66 b	0.66 b	0.52 b	0.22 b
NPK fertilization (NPK)	No fertilization	0.52 b	0.62 c	0.61 c	0.54 c	0.57 c	0.47 b	0.22 b
	PK 50%	0.50 b	0.61 c	0.61 c	0.53 c	0.55 c	0.65 a	0.32 a
	PK+50% N	0.62 a	0.72 b	0.76 b	0.76 b	0.75 b	0.66 a	0.31 a
	PK+100% N	0.64 a	0.78 a	0.82 a	0.83 a	0.81 a	0.49 b	0.22 b
p-value	PC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.842	0.395	0.105	0.012	0.005	0.053	< 0.001
LSD 5%	PC	0.05	0.05	0.06	0.05	0.04	0.11	0.08
	NPK	0.04	0.04	0.05	0.04	0.04	0.09	0.07
	PCxNPK	0.07	0.07	0.08	0.10	0.09	0.16	0.11

Table 59: Effect of preceding crops and NPK fertilization on NDVI of winter rye 2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		Vegetation Index (NDVI) winter rye			
		2020			
		DC 36	DC 51	DC 65	DC 73
Preceding crop (PC)	Fallow	0.34 c	0.31 c	0.32 c	0.40 ns
	Crimson clover	0.44 a	0.39 a	0.39 a	0.41 ns
	Field bean	0.40 b	0.35 b	0.37 ab	0.42 ns
	Oat	0.40 b	0.34 b	0.35 b	0.42 ns
	Maize	0.34 c	0.31 c	0.31 c	0.39 ns
NPK fertilization (NPK)	No fertilization	0.29 c	0.22 c	0.25 c	0.32 c
	PK 50%	0.28 c	0.23 c	0.22 d	0.30 c
	PK+50% N	0.44 b	0.41 b	0.40 b	0.48 b
	PK+100% N	0.52 a	0.50 a	0.52 a	0.54 a
p-value	PC	< 0.001	< 0.001	< 0.001	0.495
	NPK	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.152	0.019	0.630	0.805
LSD 5%	PC	0.02	0.03	0.03	0.04
	NPK	0.02	0.03	0.03	0.04
	PCxNPK	0.05	0.07	0.07	0.08

Regarding NDRE in winter wheat 2019, this tendency was shifted to heading stage DC 50 and DC 64 caused by mineral N fertilization (table A 4) and the highest NDRE level was recorded at 0.43. In 2020, in winter rye the same tendency was found (table A 5).

4. 2. 5 Chlorophyll content (SPAD) of the leaves

Legumes including crimson clover and field bean as preceding crops compared to oat and maize effectively increased the chlorophyll content (SPAD) of wheat in 2015 grown directly after the preceding crops (p value < 0.001) (table 60). Field bean and crimson clover showed the same tendency, but the maximum chlorophyll content (SPAD) of the wheat leaves was achieved by crimson clover on all four dates of measurements (table 60).

Table 60: Effect of preceding crops and NPK fertilization on chlorophyll content (SPAD) of the wheat leaves (flag leaves) in 2015, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		Chlorophyll content of wheat leaves (SPAD)			
		2015			
		15 May	29 May	05 June	16 June
Preceding crop (PC)	Fallow	50 bc	50 b	50 b	47 b
	Crimson clover	55 a	53 a	53 a	52 a
	Field bean	51 b	53 a	52 a	51 a
	Oat	47 cd	48 bc	48 c	46 b
	Maize	46 d	47 c	47 c	45 b
NPK fertilization (NPK)	No fertilization	46 c	47 c	46 c	43 c
	PK 50%	46 c	46 c	45 c	43 c
	PK+50% N	52 b	52 b	53 b	51 b
	PK+100% N	54 a	56 a	56 a	56 a
p-value	PC	< 0.001	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.119	0.094	0.013	0.023
LSD 5%	PC	2	2	2	2
	NPK	2	2	2	2
	PCxNPK	5	5	4	5

In addition, mineral N fertilization in both levels has increased the SPAD values of the wheat leaves (p value < 0.001) also on all four dates of the measurements that were carried out (table 60). However, there was an interaction between both factors (pre crop x NPK fertilization) on the two dates in June 2015 (p value 0.013 / 0.023) indicating varying effect of crimson clover in combination with NPK fertilization. Thus, the SPAD level of clover x control and clover x PK was similar to same to field bean x control and field bean x PK but significantly higher than those combinations after fallow, oat and maize (figure 28).

Further on, on 16 June 2015 after crimson clover PK treatment (without N) led to statistically same SPAD values of wheat as PK+50% N (figure 29). In contrast, the SPAD values in the PK treatment after fallow, oats and maize were significantly lower than after clover and identical to the respective control (figure 29).

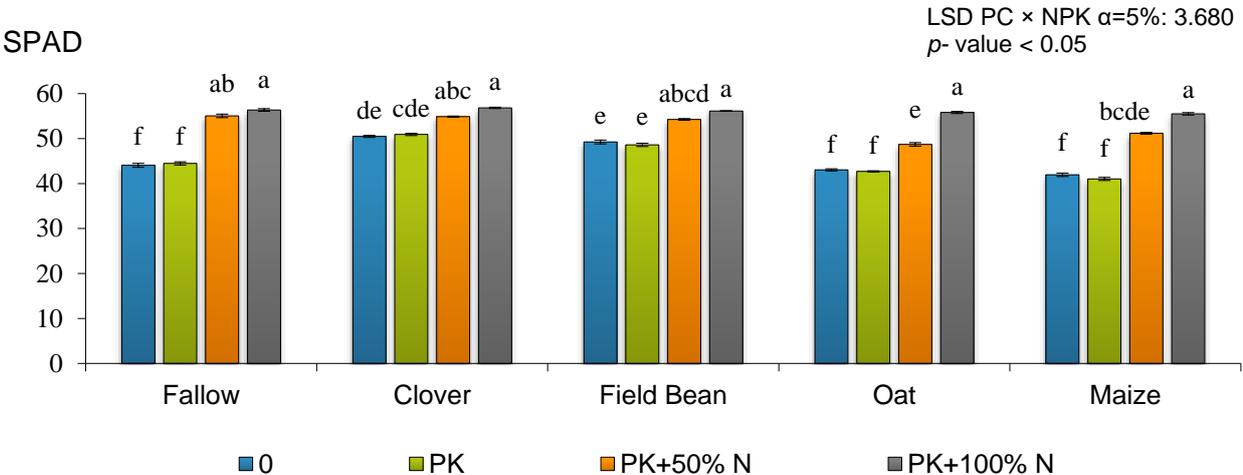


Figure 28: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on chlorophyll content (SPAD) of flag leaves in winter wheat in 05.06.2015, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

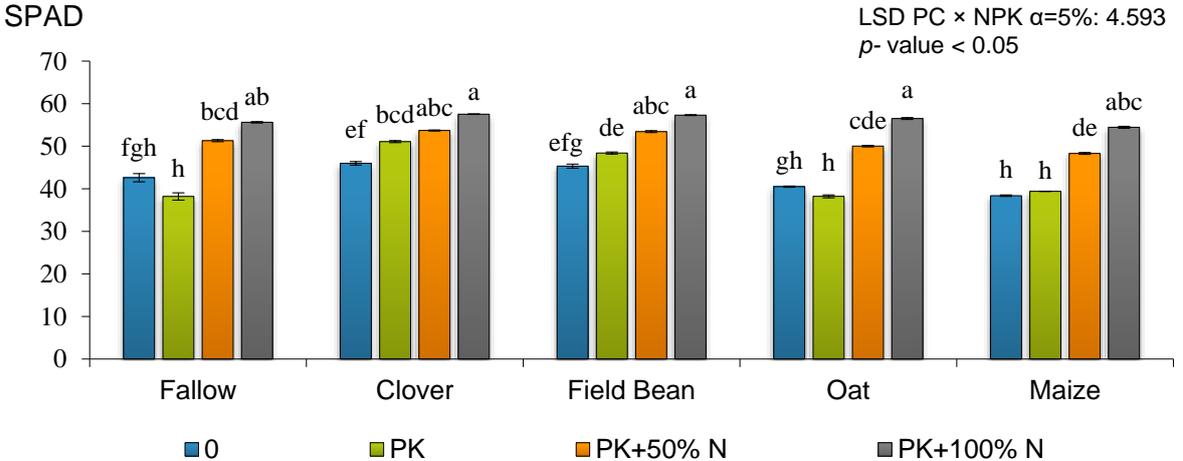


Figure 29: Interaction effect of pre-crops (PC) and mineral fertilization (NPK) on chlorophyll content (SPAD) of flag leaves in winter wheat in 16.06.2015, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

In 2016, in winter rye, in the third year of the crop rotation, smaller differences between preceding crops were observed compared to the year before (2015). The statistical analysis showed the same effect on chlorophyll content (SPAD) induced by crimson clover, field bean and oat. The same result was found on 21 June 2017 in summer barley, but no pre-crop effects were found on 11 June 2016 and 10 June 2017 (table 61). N fertilization (PK+50% N, PK+100% N) resulted in significant higher SPAD values compared to the control and PK on four out of five dates of measurement (table 61).

Table 61: Effect of preceding crops and NPK fertilization on chlorophyll content (SPAD) of flag leaves in winter rye and summer barley (2016-2017), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment; ns: not significant.

Treatments		Chlorophyll content (SPAD) of the leaves				
		Winter rye 2016			Summer barley 2017	
		13 May	25 May	11 June	10 June	21 June
Preceding crop (PC)	Fallow	48 b	48 b	47 ns	40 ns	38 b
	Crimson clover	49 a	50 a	50 ns	41 ns	39 ab
	Field bean	49 a	50 a	49 ns	41 ns	41 a
	Oat	49 ab	50 a	48 ns	40 ns	39 b
	Maize	48 b	48 b	48 ns	41 ns	40 ab
NPK fertilization (NPK)	No fertilization	45 c	45 c	44 c	41 ns	37 c
	PK 50%	45 c	46 c	43 c	41 ns	37 c
	PK+50% N	50 b	50 b	51 b	42 ns	40 b
	PK+100% N	53 a	55 a	56 a	39 ns	43 a
p-value	PC	0.037	0.011	0.367	0.987	0.033
	NPK	< 0.001	< 0.001	< 0.001	0.432	< 0.001
	PC×NPK	0.484	0.584	0.280	0.989	0.590
LSD 5%	PC	1	1	2	3	2
	NPK	1	1	2	3	2
	PC×NPK	2	2	5	7	4

The results from 2019 and 2020 are presented in table 62. The statistical analysis has shown significant higher chlorophyll content (SPAD) of the leaves in winter wheat induced by crimson clover as a green mulch (p value < 0.01) only in 2019. In contrary, in 2020 (two years after preceding crops) no significant effect of preceding crops was observed on SPAD values of winter rye (table 62).

In both years (in 2019 and in 2020) the mineral N fertilization (PK+50% N and PK+100% N) has significantly increased the chlorophyll content (SPAD) of the wheat leaves (p value < 0.01) compared to the variants not fertilized with N (table 62).

Table 62: Effect of preceding crops and NPK fertilization on chlorophyll content (SPAD) of the flag leaves in winter wheat and winter rye (2019-2020), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment; ns: not significant.

Treatments		Chlorophyll content (SPAD) of the leaves					
		Winter wheat 2019			Winter rye 2020		
		24 May	29 May	13 June	07 May	14 May	29 June
Preceding crop (PC)	Fallow	46 b	46 b	43 b	44 ns	46 ns	44 ns
	Crimson clover	52 a	50 a	49 a	44 ns	44 ns	44 ns
	Field bean	47 b	45 b	44 b	43 ns	47 ns	44 ns
	Oat	46 b	45 b	42 b	44 ns	45 ns	45 ns
	Maize	48 ab	47 b	43 b	44 ns	45 ns	45 ns
NPK fertilization (NPK)	No fertilization	44 b	42 b	38 c	39 c	39 c	42 b
	PK 50%	42 b	42 b	38 c	39 c	39 c	38 c
	PK+50% N	51 a	50 a	49 b	46 b	50 b	48 a
	PK+100% N	53 a	52 a	52 a	51 a	54 a	50 a
p-value	PC	0.004	< 0.001	< 0.001	0.919	0.164	0.813
	NPK	< 0.001	< 0.001	< 0.001	0.432	< 0.001	< 0.001
	PC×NPK	0.490	0.095	0.047	0.255	0.985	0.846
LSD 5%	PC	5	3	3	2	3	3
	NPK	4	2	3	2	3	3
	PC×NPK	7	4	4	3	6	7

4. 2. 6 Biomass yield

Winter wheat

Over the years, there is a sustainable effect of legume pre-crops on biomass yield (grain and straw) of subsequent crops within the 9th crop rotation. In the first year of crop rotation (in 2015), a significant interaction between both factors (pre-crop and NPK fertilization) was observed (p-value < 0.01). Crimson clover as green mulch and field bean improved the biomass yield to the maximum levels of 124 and 102.8 dt ha⁻¹ respectively, while oat and maize led to lower biomass yield compared to fallow. On the other hand, mineral N fertilization in both levels (PK 100%+50% N and PK+100% N) caused significantly higher biomass yields of 108.6 and 132.8 dt ha⁻¹ compared to PK 50% or control 69.4 dt ha⁻¹. The highest biomass yield was reached with 149.5 dt ha⁻¹ by crimson clover when it was accompanied by a full dosage of NPK fertilization (figure 30).

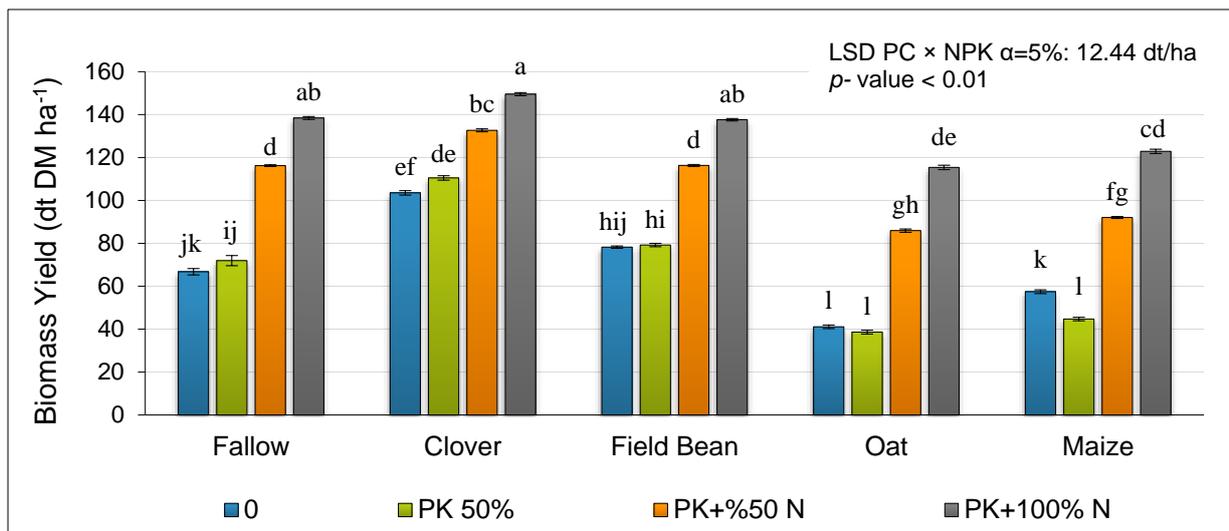


Figure 30: Biomass yields (straw + grains) of winter wheat depending on cropping systems and mineral NPK fertilization, interaction effect between both factors, LTE “BNF” Giessen 2015. Different letters indicate significant differences among the means of treatment.

In contrary to 2015, in the 10th crop rotation, in 2019, only pre-crops and NPK fertilization as main factors significantly affected the wheat biomass production. Clover mulch has caused the highest biomass yield of 170 dt ha⁻¹; followed by fallow, field bean, and maize (146.6, 145.5, and 143 dt ha⁻¹) with lower but identical levels, compared to oat with 119.4 dt ha⁻¹ (figure 31). In addition, N fertilization significantly increased wheat biomass production by applying 50% N fertilizer from 167.6 dt ha⁻¹ to 191.9 dt ha⁻¹ by 100% nitrogen application (figure 32). Although no significant interaction was observed between the main factors (pre-crops and mineral NPK fertilization), but the highest biomass yield of 202.1 dt ha⁻¹ in 2019 was achieved by crimson clover when the optimal dosage of mineral fertilization (PK+100% N) was applied (data not shown).

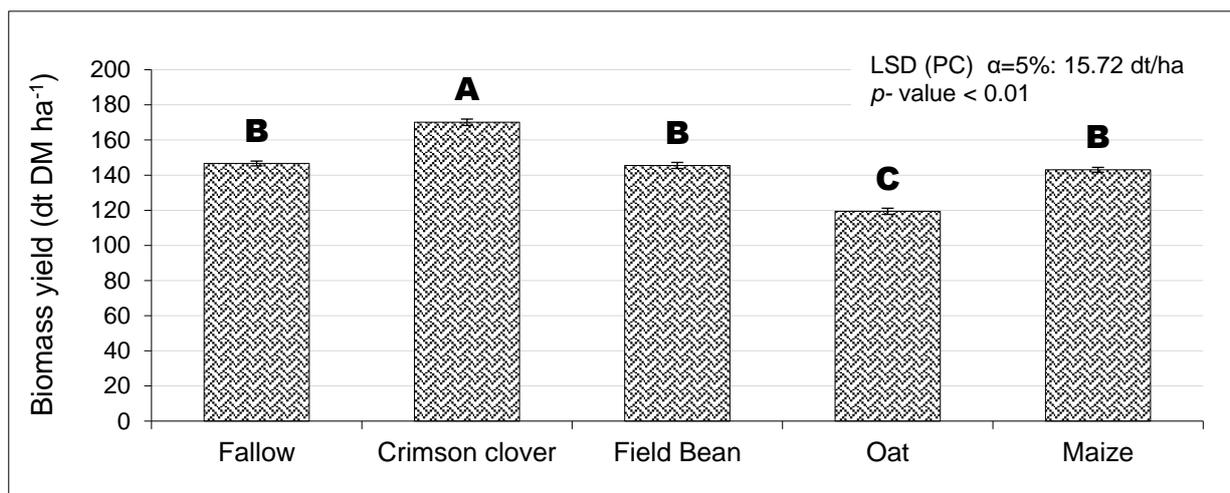


Figure 31: Effect of cropping systems on biomass yields (grain and straw) of winter wheat in 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

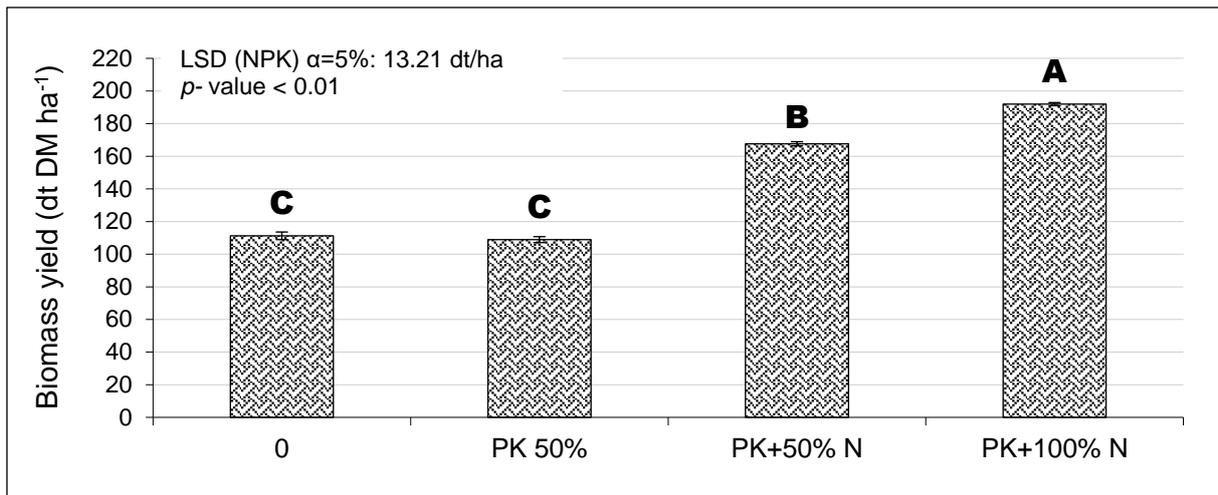


Figure 32: Effect of mineral NPK fertilization on biomass yields (grain and straw) of winter wheat in 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Winter rye

In 2016, the second year after the previous crop, only comparatively low biomass yields of winter rye were observed. In this year only crimson clover as a green mulch outperformed the biomass yield to 100 dt ha⁻¹ and significantly exceeded the yields of the other pre crops (figure 33). The lowest yield level of winter rye two year after the preceding crops was caused by fallow land (figure 33).

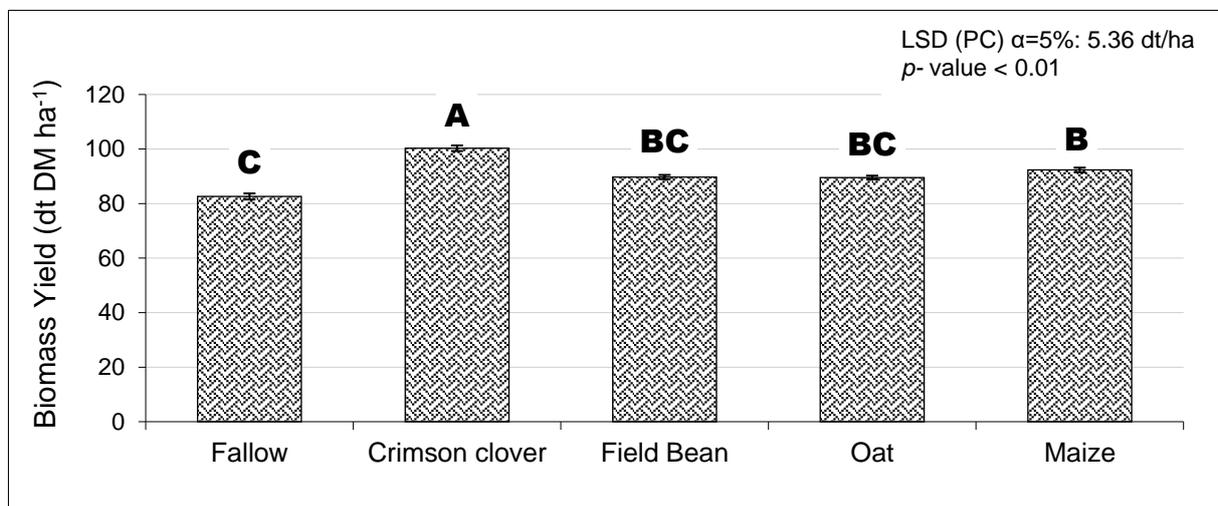


Figure 33: Effect of cropping systems on biomass yields (grain and straw) of winter rye in 2016 (mean ± SE), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

In addition, the biomass yields positively responded to the two levels of nitrogen fertilizer by increasing the yield from 106.7 dt ha⁻¹ (PK+ 50% N) to 137.9 dt ha⁻¹ (PK+100% N) compared to control (55.5 dt ha⁻¹), which received zero NPK fertilizer (figure 34).

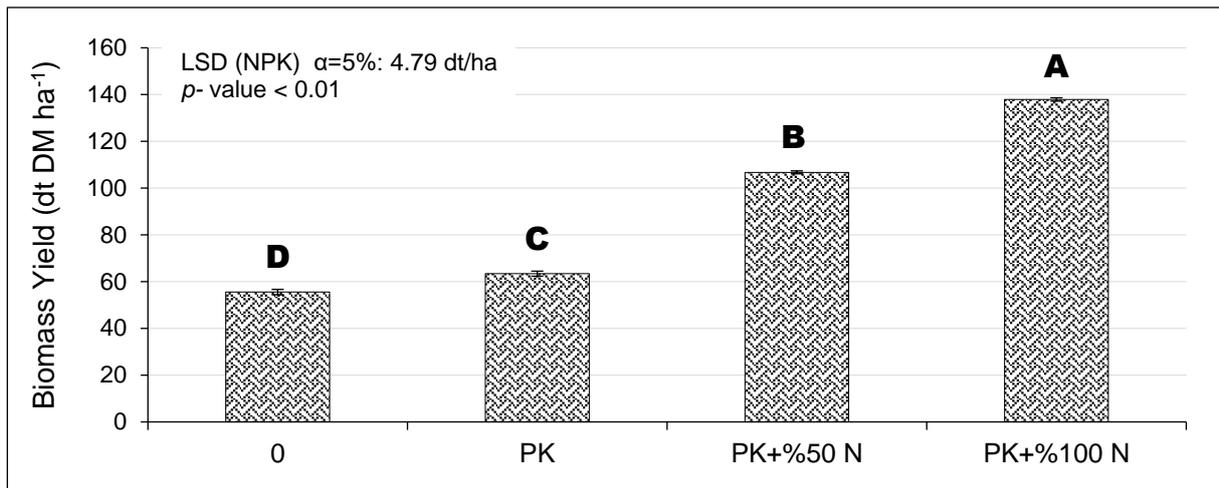


Figure 34: Effect of mineral NPK fertilization on biomass yields (grain and straw) of winter rye in 2016, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

In 2020, winter rye yields were only affected by N fertilization (p -value < 0.01), while neither legumes nor cereals as previous crops had an influence on the biomass yield of winter rye (p -value = 0.390). Zero NPK fertilization and PK 50% had lower and approximately identical levels (50.5 and 45.7 dt ha⁻¹) in biomass yield production. While PK+50% N and PK+100% N promoted this value to 87.7 and 109.6 dt ha⁻¹ (figure 35).

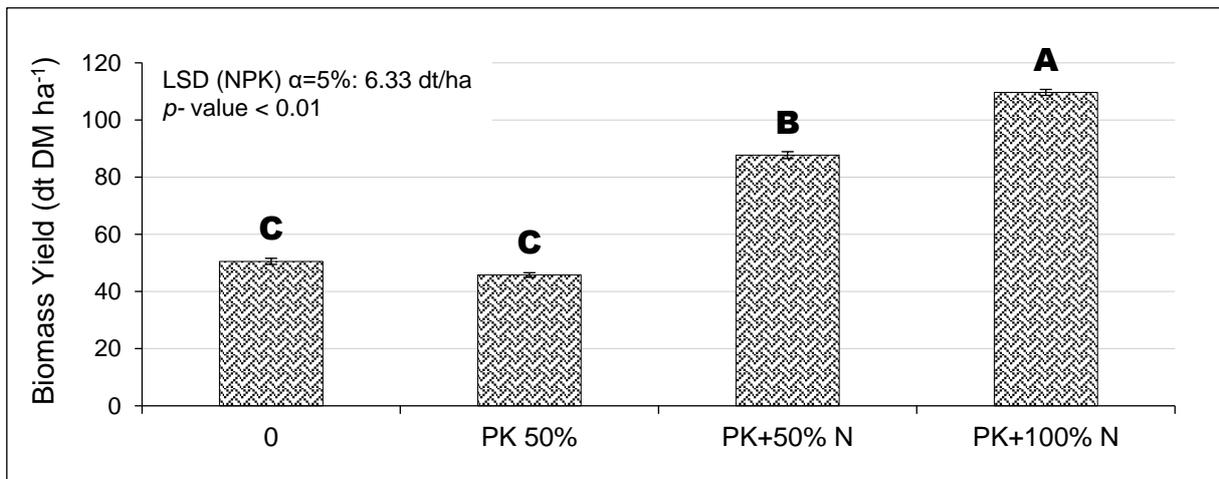


Figure 35: Effect of mineral NPK fertilization on biomass yields (grain and straw) of winter rye in 2020, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Summer barley

In the last year of the 9th crop rotation, three years after cultivating pre-crops, clear effects on the yields of spring barley could still be observed. Thus, crimson clover led to improved biomass yield production of barley reaching the highest level of 64 dt ha⁻¹. Compared to crimson clover, field bean caused lower but statistically the same biomass yield of 59.8 dt ha⁻¹. However, field bean as a legume led to the same yield level as oat and maize (56.6 and 57.7 dt ha⁻¹) and fallow had the lowest biomass yield of 51.3 dt ha⁻¹ (figure 36).

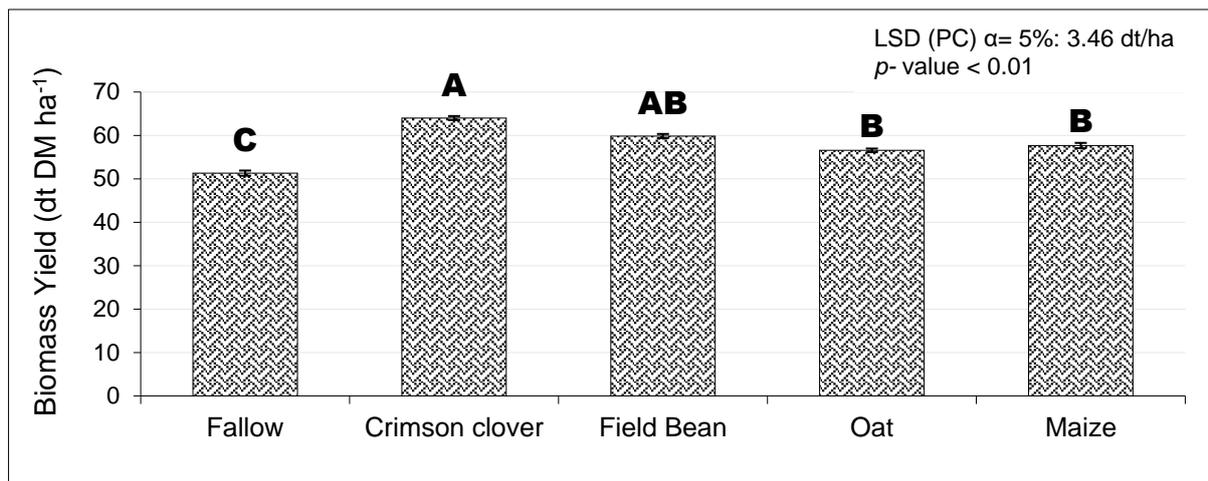


Figure 36: Effect of pre crops on biomass yields (grain and straw) of summer barley in 2017, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

The first two treatments (control and PK 50%) led to the lowest biomass yields of 46.5 and 50 dt ha⁻¹ respectively compared to those that applied with nitrogen (PK+50% N and PK+100% N) (figure 37). As expected, the highest biomass yield of 72.8 dt ha⁻¹ was achieved by a full dosage of N fertilization (PK+100% N) while decreased yield of 62.4 dt ha⁻¹ was observed by applying the half dosage of nitrogen (PK+50% N).

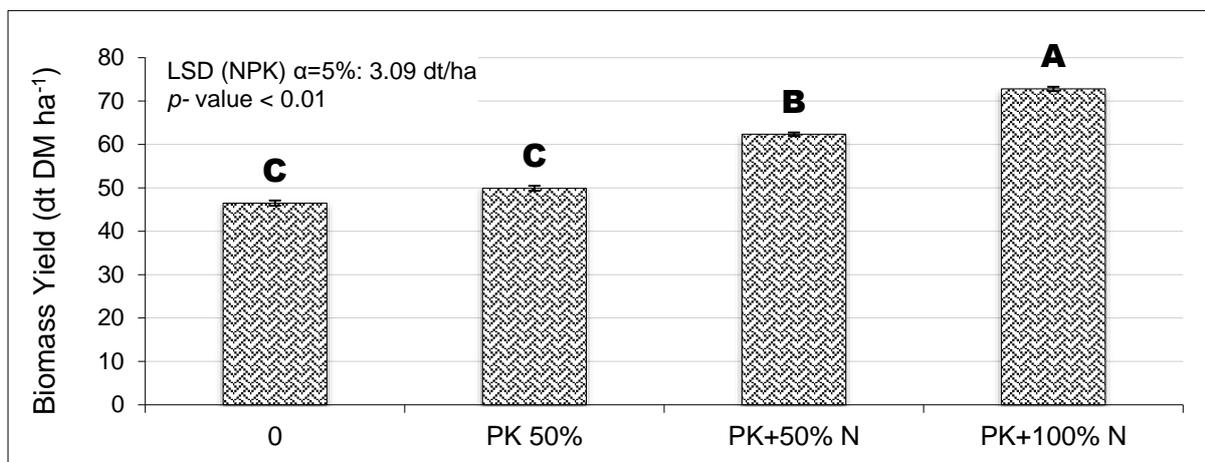


Figure 37: Effect of mineral NPK fertilization on biomass yields (grain and straw) of summer barley in 2017, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

4. 2. 7 Harvest Index (HI)

Harvest index (HI) can be defined as physiological efficiency of grain crops to convert the fraction of photo-assimilates to grain yield (Whitehead et al., 2000). From agronomic view it is characterised as the ratio of economic yield to biological yield which is achieved as final grain yield in cereals or other grain crops (Sinclair, 1998).

In the current study the HI values varied of around 0.50 to nearly 0.60 indicating quite high level of productivity of the cereals which were investigated (table 63). Thus, it was found that the HI of winter wheat in 2015 and 2019 was not significantly affected by preceding crops

within the 9th and 10th crop rotation (table 63). Despite, in both years mineral N fertilization at both levels (PK+50% N and PK+100% N) significantly increased the HI of wheat plants. So, in 2015 the application of mineral N increased the HI values from 0.55 (control) to 0.59 (PK+100% N). The same trend was observed in 2019 (table 63).

In contrast the HI of winter rye in 2016 was influenced neither by pre-crops (p-value = 0.735) nor by mineral N fertilization (p-value = 0.244). In contrary, within the 10th crop rotation (in 2020) both levels of mineral N including PK+50% N (0.59) and PK+100% N (0.61) significantly increased the HI of winter rye compared to the control (0.53).

Further on, it was found that with summer barley (in 2017) HI was significantly affected by pre-crops (p-value = 0.017) as well as mineral N fertilization (p-value < 0.01). Thus, crimson clover led to higher HI of barley (0.52) compared to control and also to maize (0.48). However, the HI increasing effect of clover mulch was statistically identical with field bean and oat (both with HI 0.50). Furthermore, mineral N fertilization at both levels (HI = 0.54) were significantly increased the HI values of summer barley compared to the control (0.46).

Table 63: Harvest Indices (HI) of the subsequent crops winter wheat, winter rye and summer barley, LTE BNF Giessen 2015-2020.

Harvest Index	Winter wheat 2015	Winter rye 2016	Summer barley 2017	Winter wheat 2019	Winter rye 2020
Fallow	0.58 ns	0.56 ns	0.48 b	0.50 ns	0.57 ns
Clover mulch	0.56 ns	0.58 ns	0.52 a	0.50 ns	0.55 ns
Field bean	0.56 ns	0.57 ns	0.50 ab	0.50 ns	0.57 ns
Oat	0.56 ns	0.58 ns	0.50 ab	0.50 ns	0.55 ns
Maize	0.56 ns	0.56 ns	0.48 b	0.49 ns	0.58 ns
p-value	0.392	0.735	0.017	0.684	0.226
LSD 5% (%)	0.021	0.031	0.025	0.022	0.033
No fertilization	0.55 b	0.57 ns	0.46 b	0.48 b	0.53 c
PK 50%	0.56 b	0.26 ns	0.45 b	0.48 b	0.52 c
PK+50% N	0.57 b	0.58 ns	0.53 a	0.51 a	0.59 b
PK+100% N	0.59 a	0.58 ns	0.54 a	0.52 a	0.61 a
p-value	< 0.001	0.244	< 0.001	< 0.001	< 0.001
LSD 5% (%)	0.019	0.028	0.022	0.019	0.029

4. 2. 8 Thousand grain weight (TGW)

TGW is an important yield component that influences the grain yield of the cereals and is influenced by numerous factors (e. g. variety, tiller density, fertilization, and climate). In the first year (wheat 2015), no significant effects were recorded by preceding crops on TGW of winter wheat (p-value = 0.071). Contrary to this, in winter wheat 2019, at the same position of wheat within the crop rotation a significant effect of pre-crops was found (p-value < 0.05). Crimson clover, field bean, maize and fallow increased the TGW of winter wheat at the same levels (50 g) but oat reached the lowest value of 48 g (table 64).

In addition, in both years (2015 and 2019) by applying mineral fertilizer (NPK), a positive tendency was found. The statistical analysis in 2015 revealed that the additional mineral N fertilization could increase the TGW of winter wheat from 52 (PK+50% N) to 53 g by using the optimal level of mineral fertilization (PK+100% N). Hence, the control plots (zero fertilization) presented the minimum level of 51 g. This effect in 2019 was in favor of split dosage of mineral N fertilization (PK+50% N), as it could increase the TGW of winter wheat to the highest level of 51 g. More to that, a significant interaction between both factors (pre-crop and NPK fertilization) was observed in 2015 (p-value < 0.01) and 2019 (p-value < 0.05) (figure 38 and 39), which was mainly induced by mineral N fertilization (table 64).

Table 64: Effect of preceding crops and NPK fertilization on TGW of winter wheat, winter rye and summer barley (2015-2020), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment; ns: not significant.

Treatments		Thousand grain weight (TGW)				
		Winter wheat	Winter rye	Summer barley	Winter wheat	Winter rye
		2015	2016	2017	2019	2020
Preceding crop (PC)	Fallow	51.5 ns	33.7 ns	45 b	49.9 a	37 ns
	Crimson clover	51 ns	34.3 ns	48.3 a	49.4 ab	36 ns
	Field bean	51.9 ns	34.2 ns	46.8 ab	49.7 a	36 ns
	Oat	51 ns	33.8 ns	46.6 ab	48.5 b	36 ns
	Maize	51.2 ns	34 ns	45.3 b	49.6 a	36 ns
NPK fertilization (NPK)	No fertilization	50.9 c	33.1 b	44.4 b	49.5 b	36 ns
	PK 50%	50.7 d	33 b	45.2 b	49.1 bc	36 ns
	PK+50% N	51.8 b	35 a	47.5 a	50.8 a	36 ns
	PK+100% N	51.9 a	35.2 a	48.5 a	48.4 c	37 ns
p-value	PC	0.071	0.483	< 0.001	0.018	0.525
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	0.590
	PC×NPK	0.002	0.456	0.290	0.024	0.882
LSD 5% (g)	PC	0.7	0.7	1.5	0.9	0.9
	NPK	0.6	0.7	1.3	0.8	0.8
	PC×NPK	1.5	1.5	3	1.8	1.9

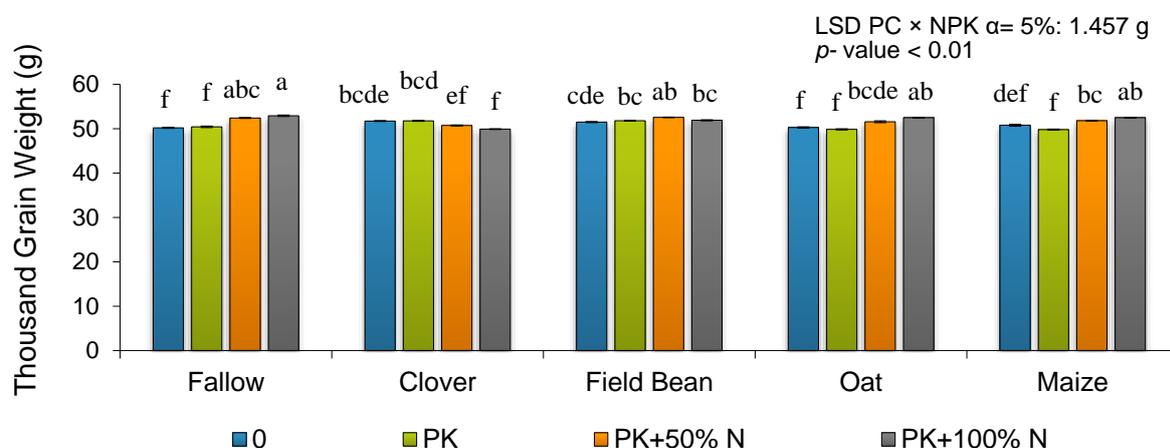


Figure 38: Effect of pre-crop (PC) and mineral fertilization (NPK) on TGW of winter wheat in 2015, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

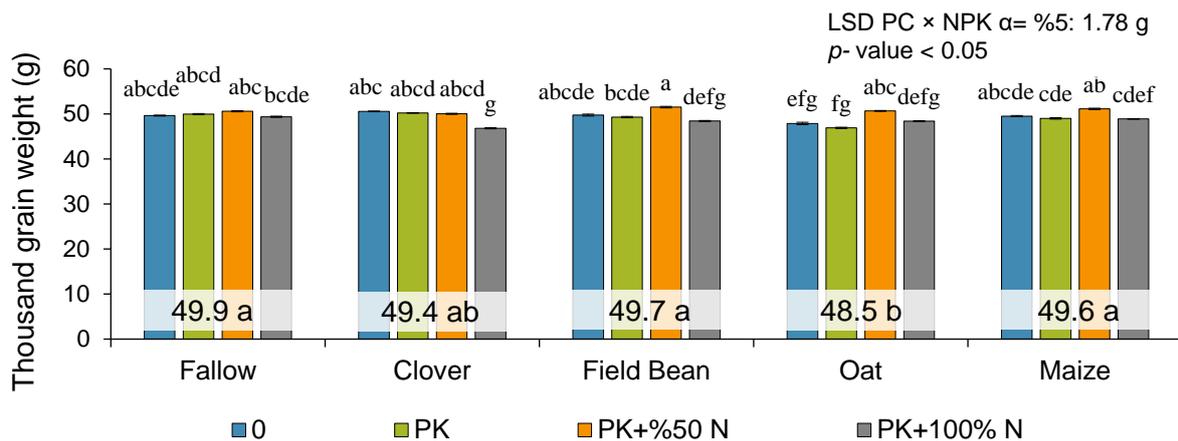


Figure 39: Effect of pre-crop (PC) and mineral fertilization (NPK) on TGW of winter wheat in 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

In winter rye 2016, the second year of the crop rotation afterwards of preceding crops, no significant effect of preceding crops on TGW was observed (p -value = 0.483). On the other hand, there was a positive response of winter rye to mineral N fertilization (p -value < 0.01). Thus, the additional N fertilization has improved the TGW of winter rye from 35 g by the application of PK+50% N to 35.2 g when the optimal levels of fertilizers were implemented (PK+100% N). So far, no interactions between treatments (pre-crops and mineral fertilization) were found (p -value = 0.456) (table 64). In 2020, winter rye was cultivated again, two years after the preliminary trials. In contrast to 2016 (crop rotation before), the TGW values of the rye in 2020 were not influenced by the previous crops or NPK fertilization.

In 2017, the fourth year of the crop rotation, TGW of summer barley was improved by pre-crops (p -value < 0.01) as well as NPK fertilizations (p -value < 0.01). According to statistical analysis, in this year crimson clover used as a green mulch led to increased TGW of summer barley to the maximum level of 48.3 g. Field bean (with 46.8 g) and oat (with 46.6) reached the same result as crimson clover while the lowest TGW value was achieved by fallow (45 g). In addition, also a significant effect of N fertilization was found (p -value < 0.01). The optimal dosage of NPK fertilization has increased the TGW of summer barley from 47.5 g (PK+50% N) to 48.5 g. No significant interactions between pre-crops and mineral fertilization observed (table 64).

4. 2. 9 Nitrogen use efficiency (NUE_{crop} and NUE_{soil})

Within the 9th crop rotation in 2015, based on 90 kg N there was a N use efficiency of 0.92 and 1.05 kg DM/kg N in oat and maize but only 1.67 kg DM/kg N in the clover mulch system, in which N use efficiency was 59 - 82% higher in clover mulch (table 65). Also, field bean as pre-crop and fallow led to higher N use efficiency of 1.33 and 1.32 kg DM/kg N compared to oat and maize. Higher N doses (180 kg N) led to lower N use efficiency values but the trend depending on pre-crop was the same; in clover 1.07 and in oat and maize 0.75 and 0.78 kg DM per 1 kg N were achieved.

In winter rye 2016, the N use efficiency of 1.53 kg DM/kg N was received from clover mulch, while the values of 1.42 and 1.37 kg DM/kg N were found in field bean and oat, respectively. Conversely, a lower level was observed in fallow (1.12 kg DM/kg N) and maize treatment (1.33 kg DM/kg N). Afterwards, the higher N levels (120 kg N) led to decrease the N use efficiency in clover (1.11 kg DM/kg N), field bean (1.02 kg DM/kg N), oat and maize treatments (1 kg DM/kg N) respectively. The value was also lower in fallow (0.91 kg DM/kg N). In spring barley 2017 based on fertilization of 60 kg N, fallow (0.80 kg DM/kg N), oat (0.87 kg DM/kg N) and maize (0.90 kg DM/kg N) achieved lower N use efficiency values compared to clover mulch (0.99 kg DM/kg N) and field bean (0.97 kg DM/kg N). Higher N doses (90 kg N) led to decrease of N use efficiency of clover mulch, field bean, fallow, oat and maize.

Table 65: N use efficiency of subsequent crops of winter wheat, winter rye and summer barley in kg/kg N within the 9th (2015-2017) and 10th (2019-2020) crop rotation, calculations are based on N uptake of grain yield followed by equation 5.

Treatments (pre-crops)	N use efficiency (NUE _{crop})									
	9 th crop rotation						10 th crop rotation			
	Winter wheat 2015		Winter rye 2016		Summer barley 2017		Winter wheat 2019		Winter rye 2020	
	90 N	180 N	60 N	120 N	60 N	90 N	90 N	180 N	60 N	120 N
Fallow	1.32	0.96	1.12	0.91	0.80	0.71	1.94	1.16	1.30	0.95
Clover mulch	1.67	1.07	1.53	1.11	0.99	0.86	2.10	1.23	1.24	0.94
Field bean	1.33	0.94	1.42	1.02	0.97	0.80	1.83	1.16	1.14	0.88
Oat	0.92	0.75	1.37	1.00	0.87	0.80	1.46	1.06	1.31	0.96
Maize	1.05	0.78	1.33	1.00	0.90	0.84	1.67	1.13	1.26	0.94

Within the 10th crop rotation, based on N fertilization of 90 kg N/ha there was the highest N use efficiency caused by clover mulch (2.10 kg DM/kg N) compared to field bean (1.83 kg DM/kg N), fallow (1.94 kg DM/kg N), maize (1.67 kg DM/kg N) and oat (1.46 kg DM/kg N). The higher N level (180 kg N) resulted in lower N use efficiency values in winter wheat 2019 across all preceding crops. In winter rye in 2020, based on N fertilization of 60 kg N/ha, NUE values of 1.24 - 1.26 kg DM/kg N in clover mulch and maize, 1.14 kg DM/kg N in field bean, 1.31 and 1.30 kg DM/kg N in oat and fallow were observed. The same trend but with smaller variation between the preceding crops was found in the treatment of 120 kg N/ha.

Regarding N use efficiency (NUE_{soil}), the result showed that under different land use management or either based on different N levels (90 and 180 kg N) clover mulch led to lower NUE_{soil} (table 66). That means under favourable cultivation system (green manure) the lower N input is needed in system to achieve the higher grain yield and biomass yield of wheat (2019) than unfavourable cropping systems (field bean straw or cereal cropping system). Based on 0 kg N, clover mulch (93 kg DM/kg N) reduced the N input for wheat grain production by 32.4% compared to maize (129 kg DM/kg N). The higher N dosages (90 kg N and 180 kg N) also reduced the NUE_{soil} values for grain and biomass production of winter wheat in the second year of the cropping system.

In 2020, based on kg N received from clover mulch (27 kg DM/kg N) and fallow land (28 kg DM/kg N) winter rye has achieved lower NUE_{soil} values in grain yield production compared to maize (35 kg DM/kg N), field bean (34 kg DM/kg N) and oat (32 kg DM/kg N), respectively (table 66). Further on, clover mulch and fallow land (54 kg DM/kg N) showed 12% reduction of NUE_{soil} in biomass yield of winter rye (2020) compared to maize (61 kg DM/kg N). Both levels of mineral N fertilizations (60 kg N ha⁻¹ and 120 kg N ha⁻¹) increased the NUE_{soil} of the different land use compared to control (0 kg N), and this trend was higher in 60 kg N than 120 kg N.

Table 66: N use efficiency of soil (NUE_{soil}) in kg yield/kg available N based on fertilizer and soil mineral N in winter wheat (2019) and winter rye (2020). The calculations are based on impact of N on total biomass yields and Nmin concentration in the soil profile (0-90 cm) followed by equation 6.

Treatments	NUE_{soil} based on grain yield and mineral N			NUE_{soil} based on biomass yield and mineral N		
	NUE_{soil} of winter wheat 2019					
	0 N	90 N	180 N	0 N	90 N	180 N
Fallow	112	68	45	230	134	88
Clover mulch	93	55	39	191	109	78
Field bean	116	65	44	236	125	85
Oat	104	59	44	217	114	83
Maize	129	62	45	271	125	86
NUE_{soil} of winter rye 2020						
	0 N	60 N	120 N	0 N	60 N	120 N
Fallow	28	39	35	54	64	57
Clover mulch	27	35	32	54	59	53
Field bean	34	39	33	63	67	55
Oat	32	37	34	65	65	56
Maize	35	39	35	61	65	57

4. 2. 10 NPK uptake by the plants

Nitrogen (N): In the second year of the crop rotation in 2015, the pre-crops (p -value < 0.001) as well as mineral fertilization (p -value < 0.001) was significantly influenced the nitrogen uptake of winter wheat. Thus, crimson clover has increased the N uptake of winter wheat to the maximum level of 160.3 kg ha⁻¹. Afterwards, field bean with 125.4 kg ha⁻¹ and fallow 119.8 kg ha⁻¹ reached the higher values compared to maize (96.5 kg ha⁻¹) and oat (80.9 kg ha⁻¹). In addition, both levels of mineral N (PK+50% N and PK+100% N) induced higher N uptake by winter wheat (p -value < 0.001). The additional mineral N increased the N uptake from 131.5 kg ha⁻¹ (PK+50% N) to 185.7 kg ha⁻¹. Hence, the lowest N uptake by winter wheat achieved by PK+50% (74.7 kg ha⁻¹) and zero fertilization (74.4 kg ha⁻¹). No interactions between treatments (pre-crops and NPK fertilization) were found (table 67).

In 2016, in the third year of the crop rotation, the same tendency in winter rye was found like in winter wheat (2015). Pre-crops (p -value < 0.001) and mineral fertilization (p -value < 0.001) promoted the N uptake by winter rye. Therefore, crimson clover (101.8 kg ha⁻¹) led to the maximum values of N uptakes by winter rye compared to fallow (79.8 kg ha⁻¹). The maximal dosage of mineral N has increased the N uptake from 102.3 kg ha⁻¹ (PK+50% N) to 152.2 (PK+100% N). Furthermore, PK 50% (56.6 kg ha⁻¹) and zero fertilization (51.1 kg ha⁻¹) obtained the minimum values of N uptake by winter rye (table 67).

Table 67: Effect of preceding crops and NPK fertilization on N uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		N uptake by the plants (kg N ha ⁻¹)		
		Winter wheat	Winter rye	Summer barley
		2015	2016	2017
Preceding crop (PC)	Fallow	119.8 b	79.8 c	58.2 c
	Crimson clover	160.3 a	101.8 a	74.8 a
	Field bean	125.4 b	92.0 b	68.8 b
	Oat	80.9 d	87.7 b	64.3 b
	Maize	96.5 c	91.4 b	65.9 b
NPK fertilization (NPK)	No fertilization	74.4 c	51.1 c	50.5 c
	PK 50%	74.7 c	56.6 c	52.7 c
	PK+50% N	131.5 b	102.3 b	70.2 b
	PK+100% N	185.7 a	152.2 a	92.2 a
<i>p</i> -value	PC	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001
	PCxNPK	0.764	0.267	0.483
LSD 5% (kg ha ⁻¹)	PC	9.3	6.5	4.6
	NPK	8.3	5.8	4.1
	PCxNPK	18.7	13.1	9.3

In 2017, the fourth year of the crop rotation, also the same effect of pre-crops on N uptake by summer barley was found (*p* value < 0.001). Therefore, crimson clover (74.8 kg ha⁻¹) increased the N uptake by summer barley to the highest level, compared to fallow (58.2 kg ha⁻¹). Field bean (68.8 kg ha⁻¹), oat (64.3 kg ha⁻¹) and maize (65.9 kg ha⁻¹) increased the N uptake of summer barley at the same levels. In addition, the maximum level of N fertilization increased the N uptake from 70.2 kg ha⁻¹ (PK+50% N) to 92.2 kg ha⁻¹ (PK+100% N) while PK 50% (52.7 kg ha⁻¹) and zero fertilization (50.5 kg ha⁻¹) obtained the lowest value (table 67).

In 2018, the 10th crop rotation began with the cultivation of the respective preceding crops, followed by wheat the following year. In order to obtain information on the biomass and nutrient yields of the preceding crops, the biomass (grain and straw) of the preceding crops (crimson clover, field bean, oat and maize) was determined this year. Accordingly, the higher N biomass yield of crimson clover of about 128.9 kg DM/ha was achieved by PK 50% while field bean achieved the higher N biomass yield of 80.5 kg DM/ha by PK+50% N. In addition, PK+100% N increased the N biomass yield of oat to the maximum level of 139.1 kg/ha DM 100%. The maximum dosage of mineral fertilization (PK+100% N) led to increase the N biomass yield of maize to the maximum level of 131.7 kg/ha DM 100% (table A 9).

Phosphorous (P): The analysis of variance revealed, that in 2015 there was a significant effect in P uptake by winter wheat among different preceding crops (*p*-value < 0.001). Crimson clover (25.7 kg ha⁻¹) has increased the P uptake by winter wheat to the maximum level and field bean (21.4 kg ha⁻¹) and fallow (21) had the same level. In addition, oat (14.6 kg ha⁻¹) has reached the minimum level. Thus, both levels of mineral fertilization were increased the total P uptake in winter wheat (*p*-value < 0.001). The optimal level of NPK (PK+100% N) led to

increase the P uptake from 23.3 kg ha⁻¹ (PK+50% N) to 29.1 kg ha⁻¹ compared to control (zero fertilization) with 14.1 kg ha⁻¹. No significant interactions between pre-crops or NPK fertilization were found (table 68).

In 2016, the third year of the crop rotation, significant effects of pre-crops on P uptake of winter rye were found (p -value < 0.001). Crimson clover (25.6 kg ha⁻¹) has increased the P uptake to the maximum level in winter rye whereas after maize (24.4 kg ha⁻¹), oat (23.5 kg ha⁻¹) and field bean (23.3 kg ha⁻¹) similar P uptake was found. Therefore, fallow (22 kg ha⁻¹) caused the lowest level of P uptake by winter rye plants. In addition, both levels of mineral N (PK+50% N and PK+100% N) were increased the P uptake. So that the optimal dosage of NPK fertilizer (PK+100% N) has increased this level from 27 kg ha⁻¹ (PK+50% N) to 35.4 kg ha⁻¹, while the lowest value was obtained by zero fertilization 15.2 kg ha⁻¹ (table 68). No significant interactions between pre-crops or NPK fertilization were found.

In 2017, the fourth year of the crop rotation, significant effects of pre-crops on P uptake of summer barley were found (p -value < 0.001). Crimson clover (15.8 kg ha⁻¹) increased the P uptake of summer barley to the maximum level. However, field bean (14.7 kg ha⁻¹) and maize (14.2 kg ha⁻¹) obtained the same value compared to crimson clover. Fallow (12.7 kg ha⁻¹) has caused the minimum level of P uptake by summer barley. Furthermore, mineral N increased the P uptake from 15.3 kg ha⁻¹ (PK+50% N) to 18.2 kg ha⁻¹ (PK+100% N). No significant interactions between pre-crops or NPK fertilization were found (table 68).

Table 68: Effect of preceding crops and NPK fertilization on P uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		P uptake by the plants (kg P ha ⁻¹)		
		Winter wheat	Winter rye	Summer barley
		2015	2016	2017
Preceding crop (PC)	Fallow	21.01 b	21.75 c	12.67 c
	Crimson clover	25.70 a	25.63 a	15.81 a
	Field bean	21.46 b	23.28 bc	14.72 ab
	Oat	14.65 d	23.46 b	13.74 bc
	Maize	17.19 c	24.37 ab	14.23 abc
NPK fertilization (NPK)	No fertilization	14.10 c	15.21 c	11.41 c
	PK 50%	13.60 c	17.00 c	12.03 c
	PK+50% N	23.30 b	27.15 b	15.33 b
	PK+100% N	29.10 a	35.43 a	18.18 a
p -value	PC	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001
	PCxNPK	0.114	0.268	0.491
LSD 5% (kg ha ⁻¹)	PC	1.64	1.60	1.56
	NPK	1.47	1.43	1.31
	PCxNPK	3.28	3.20	2.22

Potassium (K): Based on statistical analysis it can be stated, that in 2015, the second year of crop rotation there was significant effect in K uptake of winter wheat caused by different preceding crops (p -value < 0.001). Crimson clover (44.9 kg ha⁻¹) used as a green mulch increased the K uptake of winter wheat to the maximum level. Afterwards, field bean (37.9 kg ha⁻¹) and fallow (35.8 kg ha⁻¹) increased the K uptake of winter wheat at the same level and oat (39.8 kg ha⁻¹) obtained the lowest level of K uptake.

In addition, there was found a significant effect of mineral fertilization on K uptake of winter wheat (p -value < 0.001). The additional mineral N has increased the K uptake of winter wheat from 39.8 kg ha⁻¹ (PK+50% N) to the highest level of 50.2 kg ha⁻¹ (PK+100% N). Thus, no significant interaction was found between treatments (p -value < 0.057) (table 69).

In 2016, the third year of the crop rotation significant effects of pre-crops on K uptake of winter rye were found (p -value < 0.001). Therefore, crimson clover (85.6 kg ha⁻¹) has increased the K uptake of winter rye to the maximum level. Afterwards, field bean (72.6 kg ha⁻¹), maize (71 kg ha⁻¹) and oat (67.9 kg ha⁻¹) increased the K uptake of the winter rye at the same level and fallow obtained the lowest level (63.7 kg ha⁻¹). In addition, the maximal dosage of mineral N has increased the K uptake of winter rye from 81.2 kg ha⁻¹ (PK+50% N) to 125 kg ha⁻¹ (PK+100% N) (table 69). In this year, significant interaction between treatments (pre-crops and NPK fertilization) were found (p -value < 0.020). So that mineral fertilization induced the K uptake by pre-crops in winter rye (figure 40).

Table 69: Effect of preceding crops and NPK fertilization on K uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		K uptake by the plants (kg K ha ⁻¹)		
		Winter wheat	Winter rye	Summer barley
		2015	2016	2017
Preceding crop (PC)	Fallow	35.8 b	63.7 c	17.6 c
	Crimson clover	44.9 a	85.6 a	21.4 a
	Field bean	38 b	72.6 b	20.6 ab
	Oat	25.5 d	67.9 bc	19.1 bc
	Maize	28.8 c	71. b	19.6 ab
NPK fertilization (NPK)	No fertilization	24.9 c	37 d	16.5 c
	PK 50%	23.5 c	45 c	16.8 c
	PK+50% N	39.8 b	81 b	20.9 b
	PK+100% N	50.2 a	125 a	24.5 a
p -value	PC	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001
	PCxNPK	0.057	0.020	0.135
LSD 5% (kg ha ⁻¹)	PC	2.7	5.8	1.9
	NPK	2.4	5.2	1.6
	PCxNPK	5.5	11.6	2.7

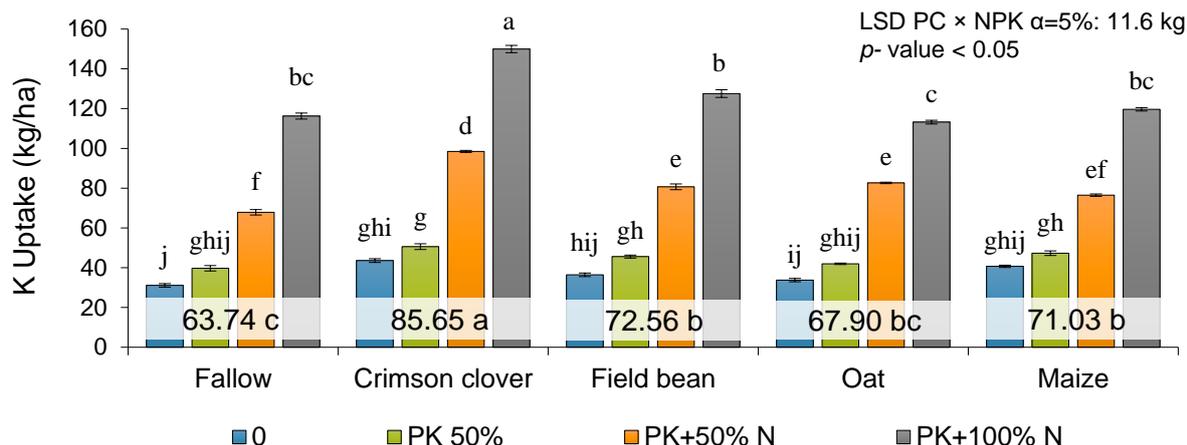


Figure 40: Effect of pre-crops (PC) and mineral fertilization (NPK) on K uptake of winter rye in 2016, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

In 2017, the last year of the crop rotation, no interactions between pre-crops and mineral fertilization were found (p -value = 0.135). Hence, the analysis of variance showed a positive response to K uptake of summer barley caused by pre-crops (p -value < 0.001). Crimson clover (21.4 kg ha^{-1}), field bean (20.6 kg ha^{-1}) and maize (19.6 kg ha^{-1}) obtained the higher K uptake values at the same level compared to oat (19 kg ha^{-1}) and fallow (17.6 kg ha^{-1}). In addition, both levels of mineral N increased the K uptake in summer barley from 20.9 kg ha^{-1} (PK+50% N) to 24.5 kg ha^{-1} (PK+100% N) compared to control (Zero fertilization) 16.5 kg ha^{-1} (table 69).

4. 2. 11 Grain quality parameters (protein and starch)

Protein content (NIRS)

The grain protein content of the investigated cereal crops winter wheat, winter rye and summer barley were subject to the influence of preceding crops (in three of five years) as well as mineral NPK fertilization (in all years which were analysed). Thus, in winter wheat 2015 crimson clover led to the maximum grain protein content of wheat compared to other preceding crops (p -value < 0.01) (table 70). Expectedly, also mineral N fertilization in both levels (PK+50% N and PK+100% N) led to increased protein contents from 10.2 to 11.7% in 2015. In winter wheat 2019 again directly after the previous crops, a similar influence by preceding crops and mineral fertilization as in 2015 was found. However, interactions between the two factors occurred in both years (figure 41 and 42). This means that the reduced N fertilization (PK+50% N) in 2015 was only effective after fallow, crimson clover and field bean, but not after oat and maize (figure 41). In contrast, both N variants led to an increase in protein content after all preceding crops in 2019 (figure 42).

In 2016, in the third year of the crop rotation significant influence of pre-crop and mineral NPK fertilization on protein content of winter rye was observed (table 70). Crimson clover (8.3%) and field bean (8.1) led to higher protein content of the grain in winter rye compared to oat, maize and fallow.

Table 70: Effect of preceding crops and NPK fertilization on grain protein content (NIRS, %) of winter wheat, winter rye and summer barley (2015-2020), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		Grain protein content-NIRS (%)				
		2015	2016	2017	2019	2020
Preceding crop (PC)	Fallow	10.21 bc	7.98 b	10.96 ns	10.52 b	8.83 ns
	Crimson clover	10.85 a	8.28 a	11.25 ns	10.91 a	8.86 ns
	Field bean	10.38 b	8.14 ab	11.28 ns	10.38 b	8.89 ns
	Oat	9.95 c	8.07 b	11.14 ns	10.38 b	9.02 ns
	Maize	9.92 c	8.06 b	11.24 ns	10.29 b	9.00 ns
NPK fertilization (NPK)	No fertilization	9.63 c	7.71 c	11.04 b	9.13 c	8.33 c
	PK 50%	9.56 c	7.64 c	10.71 b	9.14 c	8.28 c
	PK+50% N	10.17 b	8.08 b	10.93 b	11.30 b	8.94 b
	PK+100% N	11.68 a	9.00 a	12.03 a	12.42 a	10.14 a
p-value	PC	< 0.001	0.03	0.100	< 0.001	0.229
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.018	0.772	0.018	0.036	0.433
LSD 5%	PC	0.34 %	0.19 %	0.36 %	0.23 %	0.20 %
	NPK	0.31 %	0.17 %	0.30 %	0.21 %	0.18 %
	PCxNPK	0.69 %	0.38 %	0.51 %	0.46 %	0.40 %

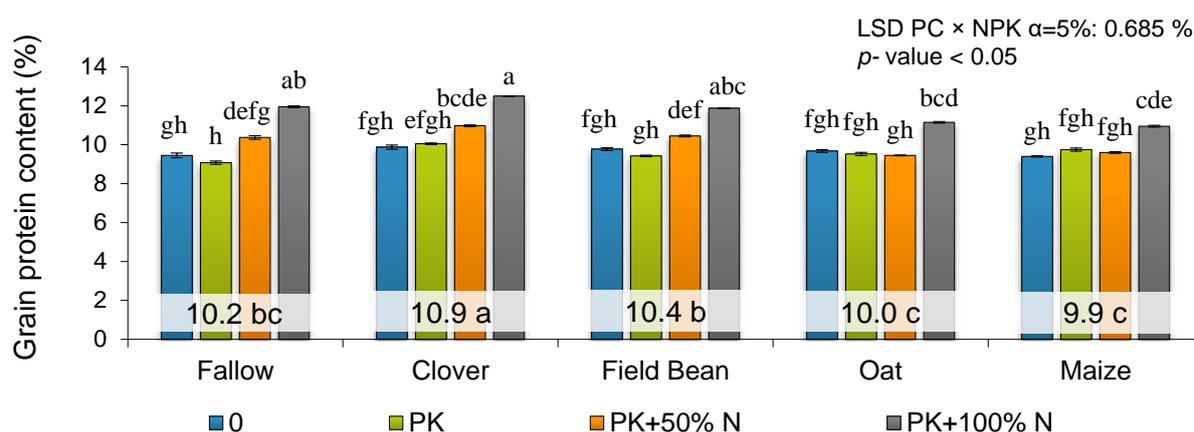


Figure 41: Effect of pre-crops (PC) and mineral fertilization (NPK) on grain protein content (NIRS) of winter wheat in 2015, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

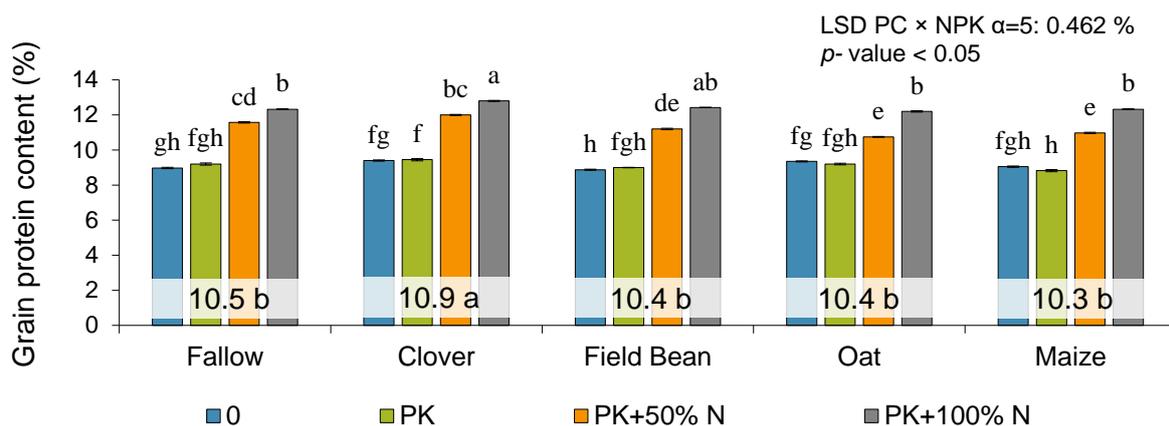


Figure 42: Effect of pre-crops (PC) and mineral fertilization (NPK) on grain protein content (NIRS) of winter wheat in 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Furthermore, there was a significant effect by NPK fertilization (p -value < 0.01) on grain protein content of winter rye caused by nitrogen supply in PK+50% N and PK+100% N (table 70). In contrary, in winter rye 2020, the grain protein content of the plants was influenced only by mineral N fertilization.

In 2017, in the fourth year of the crop rotation, no significant effect of pre-crops on grain protein content of summer barley was found (p -value = 0.100). The grain protein content ranged at about 11%. In contrary, a positive effect was observed by NPK fertilization (p -value < 0.01). The optimal dosage of NPK fertilization (PK+100% N) has increased the grain protein content of summer barley to the maximum level of 12% (table 70). But it must be considered that there was a significant interaction between both factors indicating that PK+100% N led to highest protein content in summer barley only after fallow, clover, field been and maize but not after oat (figure 43).

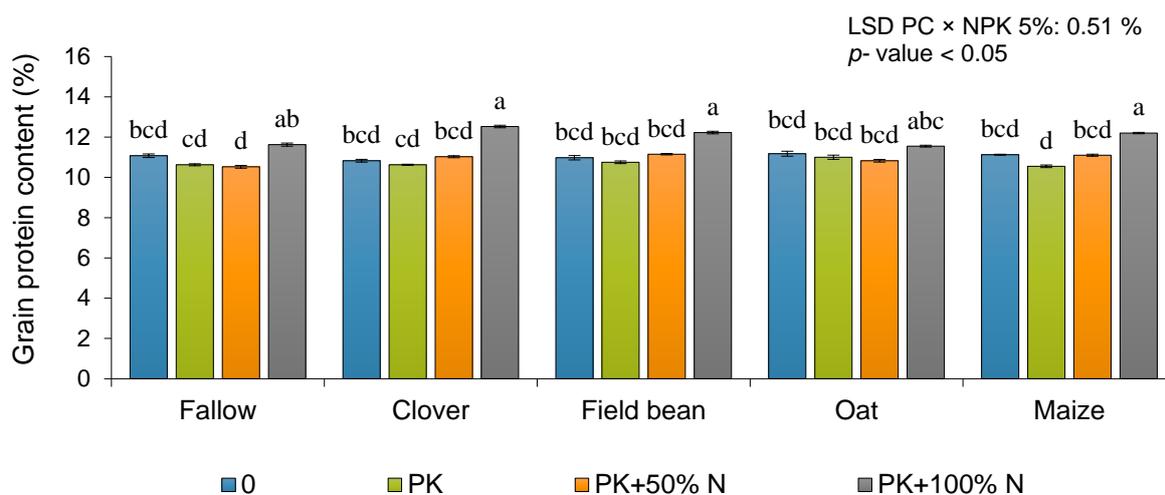


Figure 43: Effect of pre-crops (PC) and mineral fertilization (NPK) on grain protein content (NIRS) of summer barley in 2017, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Starch content (NIRS)

The starch content in the grains of the investigated cereals was at a level of about 70-71% for winter wheat as well as winter rye. In contrast, summer barley only reached about 53-55% starch due to its husk content which significantly reduces the starch content (table 71). During the whole study only in winter wheat in 2015 and in 2019 a significant pre-crop effect on starch content was observed. In these both years starch content of wheat grains has been slightly but significantly reduced by the effect of crimson clover (table 71).

Further on, there was an adverse response of starch content in the grains on mineral N fertilization, which was observed in all experimental years of the study. The starch content of the grains decreased by about one percent in all five years due to the maximum N fertilization (PK+100% N) (table 71).

Table 71: Effect of preceding crops and NPK fertilization on grain starch content (NIRS) of winter wheat, winter rye and summer barley (2015-2020), LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Treatments		Grain starch content-NIRS (%)				
		Wheat	Rye	Barley	Wheat	Rye
		2015	2016	2017	2019	2020
Preceding crop (PC)	Fallow	70.5 ab	71.7 ns	54.7 ns	70.6 a	70.7 ns
	Crimson clover	70.2 c	71.5 ns	54.7 ns	70.3 b	70.7 ns
	Field bean	70.4 b	71.6 ns	54.1 ns	70.7 a	70.8 ns
	Oat	70.6 ab	71.6 ns	53.9 ns	70.6 a	70.6 ns
	Maize	70.6 a	71.6 ns	54.3 ns	70.7 a	70.7 ns
NPK fertilization (NPK)	No fertilization	70.7 a	71.9 a	53.8 b	71.2 a	71.1 a
	PK 50%	70.6 a	71.9 ab	55.1 a	71.2 a	71.2 a
	PK+50% N	70.6 a	71.6 b	54.7 ab	70.3 b	70.6 b
	PK+100% N	69.9 b	70.9 c	53.9 b	69.6 c	69.9 c
p-value	PC	< 0.001	0.109	0.211	< 0.001	0.334
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.030	0.803	0.113	0.033	0.789
LSD 5%	PC	0.21 %	0.17 %	1.21 %	0.15 %	0.16 %
	NPK	0.18 %	0.15 %	1.02 %	0.13 %	0.14 %
	PCxNPK	0.41 %	0.33 %	1.72 %	0.29 %	0.32 %

In two years of the study (2015 and 2019) the main effects were overlapped by the interaction between both factors which expresses that the NPK fertilization was dependent on the respective preceding crops (table 71 and 72). In 2015, for example, the highest and statistically identical starch contents were achieved after all preceding crops in the absence of NPK fertilization. In the variants fertilized with N, on the other hand, wheat samples after clover and partly also after field bean had lower starch contents than after other preceding crops (table 72).

Table 72: Interaction effect between pre-crops (PC) and mineral fertilization (NPK) on grain starch content (NIRS) of winter wheat in 2015, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Starch content of grains (NIRS %) in winter wheat 2015					
Treatments	Zero NPK	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	70.75 abc	71.00 a	70.58 bcdef	69.83 gh	70.54 ab
Clover	70.65 abcd	70.33 def	70.18 fg	69.48 h	70.16 c
Field bean	70.63 abcd	70.80 abc	70.53 cdef	69.75 h	70.43 b
Oat	70.58 bcdef	70.53 cdef	70.98 ab	70.18 fg	70.56 ab
Maize	70.93 abc	70.60 bcdef	70.88 abc	70.20 efg	70.65 a
Mean	70.71 a	70.65 a	70.63 a	69.89 b	70.47
LSD PC × NPK α=5: 0.412			p- value < 0.05		

In 2019, crimson clover as preceding crop grown one year before produced the significantly lowest starch contents in the N-fertilised variants compared to the other preceding crops. In both variants without N fertilization, the starch contents after clover were also the lowest, but statistically identical to some other preceding crops (table 73).

Table 73: Interaction effect between pre-crops (PC) and mineral fertilization (NPK) on grain starch content (NIRS) of winter wheat in 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means of treatment.

Starch content of grains (NIRS %) in winter wheat 2019					
Treatments	Zero NPK	PK 50%	PK+50% N	PK+100% N	Mean
Fallow	71.38 a	71.18 abc	70.15 e	69.68 f	70.59 a
Clover	70.98 c	71.05 bc	69.80 f	69.25 g	70.27 b
Field bean	71.40 a	71.35 a	70.33 de	69.60 f	70.67 a
Oat	71.03 bc	71.18 abc	70.50 d	69.70 f	70.60 a
Maize	71.28 ab	71.25 abc	70.58 d	69.65 f	70.69 a
Mean	71.21 a	71.20 a	70.27 b	69.58 c	70.56
LSD PC × NPK α=5%: 0.29			p- value < 0.05		

Besides protein and starch, the wheat grains were also tested for other quality parameters like volume weight, sedimentation value and wet gluten. In these studies, it was found that pre-crops as well as NPK fertilization led to increased values in specific weight, sedimentation value as well as wet gluten of wheat. Most of these parameters were improved by crimson clover used as a green mulch or by the maximum level of NPK fertilization (PK+100% N). For example, crimson clover led to higher specific weight of 74.6 kg hl⁻¹, increased sedimentation volume of 37.6 ml as well as higher wet gluten content of 21.4% in winter wheat in 2015. In addition, the optimal level of NPK fertilization (PK+100% N) led to maximum specific weight (74.9 kg hl⁻¹), sedimentation volume (42.7 ml) and wet gluten content (23.4%) in the same year in winter wheat (table A 6 and A 7).

In contrary in 2019, the specific weight of winter wheat grains was at the same level after crimson clover, field bean and fallow but higher than oat and maize, respectively. Also, mineral N (PK+50% N and PK+100% N) significantly increased this parameter to 76.6 and 77.2 kg hl⁻¹ compared to control (74.1 kg hl⁻¹) (table A 6). In that year, crimson clover led to significantly increase of sedimentation (37.4 ml) and wet gluten (24.5%) values compared to field bean, oat, maize and fallow. In addition, the optimal dosage of NPK fertilization has improved these values to 47.4 ml and 27.9 %, respectively (table A 6 and figure A 1 to A 5).

Further on, in winter rye 2016 the specific grain weight was significantly affected by NPK fertilization. Thus, the optimal dosage of NPK fertilization (PK+100% N) led to higher specific weight of winter rye with about 74 kg hl⁻¹ compared to control and PK+50% N. Whereas, in 2020 neither preceding crops nor mineral NPK fertilization significantly increased the specific grain weight of winter rye (table A 7).

5 Discussion

5. 1 Effect of preceding crops/rotational crop diversity and mineral NPK fertilization on soil parameters

5. 1. 1 Mineral nitrogen (NO₃⁻)

The mineral nitrogen in the soil consisting of ammonium (NH₄⁺) and nitrate (NO₃⁻) can be directly taken up and utilized by the plants and is subject to strong temporal dynamics in the soil depending on the conditions. Important factors influencing the formation and dynamics of Nmin (NO₃⁻) are soil type, land use (including crops, tillage, and fertilization), temperature and precipitation.

In order to take the entire root depth into account, the Nmin (NO₃⁻) content is measured at a depth of 0-90 cm but only in four out of seven measurements in this study. In contrary, in 2015 only 0-40 cm and in September and November 2018 only 0-60 cm were investigated. Consequently, these measurements also allow a (limited) explanation regarding the impact of the variants on Nmin (NO₃⁻) in the soil (table 74).

Table 74: Nmin (NO₃⁻) values (kg ha⁻¹) of the soil in the 9th rotation: 2015 (one year after preceding crops) and 2016 (two years after preceding crops) and in the 10th rotation: 2018 in the year of preceding crops cultivation, 2019 one year after pre crops.

9 th crop rotation			10 th crop rotation					
2014	2015	2016	2018		2019		2020	
Pre crops	Sep.	Sep.	Pre crops	Sep.	Nov.	Mar.	Oct.	Apr.
	after wheat	after rye		after PC ¹	after PC ²	in wheat	after wheat	in rye
	0-40 cm	0-90 cm		0-60 cm	0-60 cm	0-90 cm	0-90 cm	0-90 cm
	1	2		3	4	5	6	7
Fallow	38 c	93 c	Fallow	49 a	75 b	44 b	61 b	78 ns
Clover	54 a	115 a	Clover	35 b	103 a	80 a	80 a	88 ns
Field bean	48 ab	109 a	Field bean	30 bc	57 c	46 b	70 ab	68 ns
Oat	51 ab	105 ab	Oat	24 c	40 d	34 c	75 a	81 ns
Maize	45 bc	97 bc	Maize	25 c	53 c	41 bc	64 b	73 ns

¹⁾ Two weeks after harvesting preceding crops.

²⁾ Three weeks after incorporation of pre crops residue into the soil.

The Nmin (NO₃⁻) values of the years 2015 and 2016 are not comparable with each other, as different soil horizons were investigated. Nevertheless, it can be stated that a similar effect was observed in both years indicated by higher Nmin (NO₃⁻) levels after clover mulch (similarly high after field bean and oats) but lower Nmin (NO₃⁻) levels after fallow. The results show that the cultivation of wheat and rye as pre crops did not equalise the different Nmin (NO₃⁻) values caused by the pre crops. Thus, it is supposed that the previous crops have created a high N pool, which has not yet been used up after two years.

The high Nmin (NO₃⁻) values after the harvest of winter rye in 2016 suggest a low N uptake by the rye plants. The N uptake of the rye was improved by optimal N fertilization, because the

NPK variants had lower Nmin (NO_3^-) values after the harvest of the rye than the control. This relationship is attributed to the higher biomass yields (higher N uptake) of the rye due to N fertilization.

In 2018, the Nmin (NO_3^-) analysis was carried out both in September (directly after the harvest of the preceding crops) and in November (two months after the harvest of the preceding crops). In September, the level of Nmin (NO_3^-) values was relatively low (25 - 49 kg N/ha). Two months later, the Nmin (NO_3^-) values increased to double the September values with the highest level in clover (103 kg N). This effect is attributed to the fact that the biomass incorporated into the soil (incl. roots) was increasingly mineralised till November. This mineralization of the organic mass led to the increase in Nmin (NO_3^-) values, especially in the clover variant. This hypothesis is also supported by the fact that the Nmin (NO_3^-) values in 0-30 cm were considerably higher than in 30-60 cm. This suggests that the mineralization processes starting in the topsoil layer, where most of the organic matter is located.

According to the results mentioned above, and consistent to the first hypothesis, the mineral nitrogen content of the soil profile under different cultivation system was significantly higher caused by legume crops especially clover mulch after winter wheat in 2015 within the 9th crop rotation than fallow land as expected. This potential effect can attribute to the capacity of biological nitrogen fixation among legumes. On the other hand, two different legumes crimson clover and field bean presented different Nmin (NO_3^-) amount although both have N fixation capabilities (Finney et al., 2016; White et al., 2016). This effect lies in the amount and type of organic substances between these two legumes. Thus, crimson clover contributes to the Nmin (NO_3^-) with the whole biomass generated through the growing season. In contrary, field bean contributes only with root and straw biomass excluding the seeds to the N pool in the soil, which makes field bean not efficient enough for providing available nitrogen in the soil compared to clover mulch.

It has to be considered that nitrogen rich residues of legume crops break down gradually, especially in clay soils, therefore nutrients are released slowly. So, the nitrogen concentration residue from field bean cannot sufficiently compared with crimson clover as green mulch through the biomass incorporation in the first of four years crop rotation. As the rotation turns around every four years in cultivating system, the Nmin (NO_3^-) provided by preceding crops over mineralization of the plant residues undertakes mainly through the topsoil in the first year after soil incorporation. In several other studies was also shown that green manure consisting of legumes as pure stands or as mixtures can provide more N at early growth stages than non-legumes (Brennan et al., 2013; Kramberger et al., 2014; Finney et al., 2016; Tribouillois et al., 2016; St. Luce et al., 2016).

Accordingly, within the 10th crop rotation in November 2018, in the first year of crop rotation when crimson clover was incorporated into the soil the Nmin (NO_3^-) level was increased additionally by mineral N fertilization (PK+50% N and PK+100% N). Therefore, in the second year of crop rotation in March 2019 higher Nmin (NO_3^-) concentration was found in the subsoil. This effect can be associated with the nitrogen leaching.

Despite the fact that implementing winter wheat in the first and winter rye in the second year after legumes can reduce the mineralized nitrogen in upper and subsoil by fast growth from full tillering to anthesis and by nitrate scavenging ability from subsoil (Haberle et al., 2006; Kaspar et al., 2012). Also, in other studies it was declared that N losses caused by preceding crops or by large N residues can be reduced by the subsequent crop due to its large autumnal N uptake (Benincasa et al., 2010) or by high root depth penetration rate (Dresboll and Thorup-Kristensen, 2014).

Further on, higher dosage of mineral NPK fertilization has increased the N_{min} (NO_3^-) concentration of the soil during the second and third year of the crop rotation after cultivating preceding particularly by increasing the root biomass. The result showed that the additional mineral N fertilization in winter wheat may increase mineralizable soil mineral N by increasing both the amount of crop residues and their N concentration. This result is accordance with Silgram and Chambers (2002) and Glendining and Powlson (1995) who found similar results.

5. 1. 2 Total nitrogen (Nt), total nitrogen bound (TNb) and dissolved organic nitrogen (DON)

The total available nitrogen in the soil consists of organic and inorganic forms. Therefore, the inorganic form of N comprises soluble forms (e. g., NO_2^- and NO_3^-), exchangeable NH_4^+ , and clay-fixed nonexchangeable NH_4^+ (Stevenson, 1986). It can be acquired by supervision of organic nitrogen fractions, ammonia, nitrate, nitrite individually or the complex components. In agro-ecosystems or natural environment the parameters of soil organic carbon (SOC) and total nitrogen (Nt) are characterized as prior requirements or boundaries of soil fertility.

The results of total nitrogen (Nt) supported the second hypothesis, in which the cultivation systems with a proportion of 25% legumes within the crop rotation have led to a significant increase in the Nt and TNb concentration of the topsoil in the current experiment. This effect was highest in clover mulch and lowest in fallow land. Both terms of N input, preceding crop and mineral NPK fertilization prompt to increase the soil N accumulation over the time. Hence, legumes mainly clover mulch and field bean showed a high performance of biological nitrogen fixation within the 9th and 10th crop rotation.

Soil organic matter pools have a significant control on N regulations and dynamics in legume-based cropping systems and distinguishing the management of N to optimize the soil fertility and yield production. To gain optimal yields and N regulation in legume-fertilized management systems, the interactions between microbial activity, biological N fixation and resources of the soil organic matter must be taken into account. Furthermore, the appropriate utilization of biological N fixation strategy can strengthen the nitrogen use efficiency in the terms of N losses and decline the N requirements in the management system (Drinkwater et al., 1998; Drinkwater and Snapp, 2005).

Accordingly, increasing the net nitrogen in the topsoil results in higher biomass production, which provides more crop residues in the cultivating system. Hence, through the mineralization of organic substances more available nitrogen will be provided for the subsequent crops. This will initiate to increase the populations of soil microorganisms and their activity which in turn

can ease the procedure of crop residue decomposition and mineralization of the organic matter. Other studies also showed that legume-based cropping system may have the privilege of N cycling regulations (Drinkwater et al., 1998; Unkovich & Pate, 2000; Ross et al., 2008). Alternatively, if the accumulation of the soil organic matter (SOM) increases by legume-based management system over time, N mineralization from SOM pools may suppress biological N fixation (Waterer and Vessey, 1993).

In the current study it was found that the crop rotation with 100% cereal compared to 75% cereals + 25% legume crops (only in case of clover mulch not in case of field bean) has not efficiently increased the Nt content of the topsoil due to the quantity and quality of the left residues by each pre crops on the soil surface. Due to the removal N sources of the grains which were not turned back to the soil, field bean, oat and maize showed the same tendency of total nitrogen in the soil over the years, although this trend was higher than after fallow land. Other studies also declared that the substitution of fallow land with preceding crops or cover crops is a suitable strategy in post-harvest management system for maintaining the inorganic N fractions (McCracken et al., 1994; Drinkwater and Snapp, 2005). Also, Clark et al. (1998) who carried out an eight-year investigation found a minimal N variation under the green mulching systems by the application of inorganic N fertilizer, compared with conventional management. Further on, in other long-term study conducted by Drinkwater et al. (1998) it was reported that green manure provided approximately half of the applied N in the top horizon of the soil compared to conventional system. Indeed, the conventional systems demonstrated a lower NUE even by the application of mineral N compared to the green mulching system. The results obtained from both studies declaring the close positive relationship between N accumulation in green mulching system and soil organic carbon.

Additionally, the surplus N from mineral NPK fertilization at both levels (PK+50% N and PK+100% N) significantly increased the Nt of the topsoil in the current experiment over the time. This effect is mainly attributed to the higher root biomass and the higher N content of the biomass in the soil, which was triggered by the mineral N fertilization. Presumably, this has also promoted those soil microorganisms that metabolize more nitrogen. In the trial evaluated, this process has been accomplishing for almost four decades, so that this long period was sufficient to increase the accumulation of Nt in the soil. Similar results have been reported from other LTEs (Blair et al., 2006a; Giacometti et al., 2013; He et al., 2015).

The dissolved soil organic matter (DOM) is of great importance for the transport of large amounts of carbon, nitrogen and phosphorus, as well as for the availability and relocation of trace metals and contaminants. With regard to nitrogen, the proportions of DOM are determined by DON and TNb, which play a role above all in water analysis, among other things for determining water quality. However, knowledge of the DON and TNb values in arable soils can also contribute to a deep perception of the N dynamics in the soil.

Regarding the total nitrogen bound (TNb) in the own study there was a trend of increasing values under clover mulch and mineral NPK fertilization within the upper layer of the soil but partly also down to the soil profile. In both TNb measurements (2018 + 2019), the highest values were observed in 0-30 cm, half a year after clover mulch. In March 2019, TNb values in clover mulch variant were significantly increased in the entire soil profile (0-90 cm) compared

to all the other treatments. This observation suggests that significantly more N compounds were accumulated in the clover mulch variant and distributed throughout the soil profile. It can be considered that TNb includes various N sources. These include ammonium, nitrites, nitrates and organic N compounds that are released during the degradation of organic matter in the soil. It is presumed that significantly more organic and mineral N compounds were released from the biomass of the clover mulch, which were measured as TNb values in the soil. However, nitrate leaching probably accounted for the largest proportion of N compounds measured in the entire soil profile.

In contrast, the effect of NPK fertilization was not so clear. Only in 2019 were higher values measured in the topsoil layer as a result of N fertilization (100 % NPK). In the previous year, however, there were no differences between the control and the variant with 100 % N. Therefore, higher TNb values after mineral N fertilization in March 2019 (wheat), hence, are primarily also attributed to water-soluble N compounds (nitrate).

Also, dissolved organic nitrogen (DON) of the soil was affected by clover mulch and mineral N application. Both variants showed an increase trend in DON of the upper soil in 2019 in winter wheat cultivated directly after the pre crops when the residues were incorporated into the soil. In addition, the higher N level by N fertilization to pre-crops in 2018 led to higher DON values. According to our hypothesis the higher DON values caused by clover mulch or N fertilization lies in the fact that increased organic matter and its degradation provide more available nutrients to the soil microbes which are processed into dissolved organic fractions (Jones et al., 2005; Van Hees et al., 2005). These compounds include numerous organic N compounds (e. g. urea, amino acids, amino sugars, nucleic acids, humic substances) released from exudates from bacteria, macrophages and degradation products of plant residues and soil animals. According to van Kessel et al. (2009), a distinction is made between hydrophobic and hydrophilic dissolved N compounds, which can also be characterised by a different molecular weight (HWM vs. LMW compounds). In arable soils, hydrophilic DON compounds seem to be the most common (van Kessel et al., 2009).

Overall, it can be stated that the higher TNb and DON values found in the own study, which occurred after clover mulch or N fertilization, are attributed to the organic (amino acids, amides, alkaloids, nucleic acids) and mineral (nitrates) N compounds released from clover mulch residues. The mineral N fertilization presumably contributed to increase in these values via the direct supply of mineral N or through the formation of nitrate (nitrification). It is concluded that the DON and TNb levels are not only considered as indicators of higher biological activity in the soil, but also as a starting point for leaching, translocation and possible environmental pollution by these compounds. The latter point, however, depends on the amount of soil colloids and on precipitation.

5. 1. 3 Total carbon (Ct), total organic carbon (TOC), dissolved organic carbon (DOC), C/N ratio

Total carbon (Ct)

Total carbon includes both organic and mineral carbon. However, in the topsoil from 0-30 cm and below, there are no carbonates and thus no mineral carbon at our site. For this reason, the Ct can be equated with the SOC.

A total of seven Ct analyses of the soil were carried out during the entire investigation from 2015 to 2020. In five of these tests, higher Ct values were found in 0-30 cm after clover mulch and field bean, but these differences were only significant on two of seven sampling dates (October 2019, April 2020). This result shows that it can be presumed with a certain probability that a Ct increase in the topsoil could have taken place due to legumes. However, it was not possible to provide clear evidence of this, as the differences between the mean values were not significant enough. In the subsoil (30-90 cm), no significant change in Ct values due to previous crops was observed in any of the seven studies.

This inconclusive result also shows that Ct/SOC determinations of the soil should be carried out at regular intervals and over a long period of time. Because of their long duration, LTEs are a valuable basis for this.

In contrast, numerous positive results on the influence of legumes on SOC/Ct levels can be found in the literature. Thus, Wu et al. (2017) declared an enhancement of C storage in the soil profile (0-100 cm) under legumes, which was directly addressed to the increase of above ground biomass. It is acknowledged that the N quantity in the soil is important to build up the SOC, as it also proved in further studies (Christopher & Lal, 2007; Resh et al., 2002).

Similar result was found by Zhao et al. (2014), who explained a robust relationship among legumes and soil properties, which is mainly addressed to the C and N content of the soil. Other studies recognized a possible effect of organic amendments like cover crops and the management of crop residues integrated with management practices resulting in increased SOC stocks (Aguilera et al., 2013; Blanco-Canqui 2013; Kätterer et al., 2013).

This is noteworthy to consider that the cover cropping system not only increase the soil organic carbon through leaving crop residues and root exudates into the soil, but also, they contribute to reduce the soil erosion by providing surface layers during and after cultivation. This aspect of cover crops on soil management have been reported in studies from Wang et al. (2015), Gonzales-Sanchez et al. (2012), Liu et al. (2014) and Lu (2015).

Referring to the impact of mineral NPK fertilization in the own study, a significant increase in Ct content in the topsoil (0-20 cm and 0-30 cm) was observed in five of total of seven studies (years), which was attributed to the direct and/or indirect effect of the mineral nitrogen. There are several possible reasons for this result. It is presumed that nitrogen has influenced several physical, chemical and biological properties of the soil. Thus, more mineral N input results in higher biomass production and subsequently in more organic matter availability in the upper soil. Based on this, the more organic C builds up the soil structure and increases the contribution of the soil microbiome and their activity.

Other studies also support our result regarding the N effect on Ct/SOC (Kätterer et al., 2012). This is explained mainly by increased biomass yields contributed to the increases of C inputs from crop residues as well as root remnants after harvesting crops (Christopher and Lal, 2007; Hijbeek et al., 2017; Lal, 2008b). Also, Alvarez (2005) found that SOC storage increased by 2 kg C/ha for each cumulative kg of N/ha applied. Kätterer et al. (2012) have analysed LTEs under Nordic conditions where they achieved the same result of 1 to 2 kg C ha⁻¹ year⁻¹ in the topsoil (0–20 cm) for each kg of nitrogen application. These results from long-term trials clearly show that SOC is increasing with the rate of N fertilizer applied.

Similar results were observed in other studies like the Askov long-term fertilization experiment (Schjonning et al., 1994), the Broadbalk experiment in Rothamsted (Johnston et al., 2009; Blair et al., 2006b) and the static fertilization experiment in Bad Lauchstädt (Blair et al., 2006a). In addition, increases in soil organic matter N were observed in 29 of total 34 long-term experiments reviewed by Glendining and Powlson (1995).

Total (TOC) and dissolved organic carbon (DOC)

The total organic carbon (TOC) represents a sum parameter of the total organic carbon compounds in a water or soil sample which includes purgeable and non-purgeable organic carbon. The latter is subdivided further into dissolved organic carbon (DOC) and particulate organic carbon (POC). Although TOC plays a role primarily in water analysis, where it is used to determine the purity of a water sample, it is also essential for soil research because it is considered as a sensitive parameter for soil fertility and is therefore recommended for evaluating the fertility of arable soils (Brejda et al., 2000).

Dissolved organic carbon (DOC) which represents a part of the TOC in the soil, is very active and mobile and of great importance for the C cycle. Sources of DOC include plant residues, organic wastes, excreta of soil animals, root exudates (Rasse et al., 2005; Bais et al., 2006; Mueller et al., 2013) and the products of microbial degradation of organic matter (Jastrow et al., 2007; Fornara, and Tilman, 2008; Schmidt et al., 2011). The management of crop residues and organic matter into the soil provides more substances to feed the soil living microorganisms and encouraging to increase their activity and necromass accumulation in the soil (Eisenhauer et al., 2010; Liang et al., 2011). However, this progress takes place in a certain circumstance to increase the amount of soil microbial communities. The higher application of crop residues increases the degradations of organic matter and reduces the carbon storage due to metabolic potential of the microbiomes (Manzoni et al., 2012) or even increasing the decomposition rate of sequestered carbon (Fontaine et al., 2007). In addition, the humification and decomposition of organic matter provides high-molecular humic acids and low-molecular fulvic acids, among other compounds, which make up an essential part of the DOC.

The current investigation has shown that TOC and DOC reacted positively to both test factors, preceding crops as well as mineral NPK fertilization. It was also found from DOC values that the optimal dosage of NPK fertilization has a synergistic effect on mineralization of the crop residues in the topsoil. This effect was steady higher in clover mulch and oat compared to fallow land. In addition, it was noticed that this effect only confines to the first layer of the soil (0-30 cm) and not in deeper soil horizons.

Interestingly, preceding crops which were cultivated every fourth year have increased the TOC of the topsoil determined in two years (2018 and 2019) compared to fallow land. The ranking was crimson clover = oat = field bean = maize > fallow land which means on the other hand that only on fallow land the TOC was reduced significantly. As expected, the TOC values followed a vertical gradient and decreased significantly in the three layers investigated. This gradient is possibly to be related to root density (and root residue degradation products), which also decreases vertically. In 2019, the pre-crop-related TOC differences were evident in both the topsoil and the 30-60 cm layer. Therefore, it can be presumed that there is also a relevant root density in the 30-60 cm layer that contributed to this effect. In the 60-90 cm layer, in addition, the TOC contents were very low and no longer differed from the fallow variant.

Beside pre crops, positive response was observed by mineral N input (PK+50% N and PK+100% N) as well. In confirmation with our hypothesis mineral N fertilization has improved the TOC of the topsoil significantly which was particularly the case by the application of the higher N dosage. This effect is presumably related to the root biomass promoted by the N fertilization increasing N accumulation in the root residues and in the microfauna. There is still widespread opinion that the use of inorganic N fertilizer speeds up the decomposition of soil organic matter, thereby reducing the possibility for C sequestration (Kätterer et al., 2012). The role of inorganic N fertilizer on soil organic matter changes is still discussed today (Khan et al., 2007; Reid, 2008; Mulvaney et al., 2009) and needs further investigations.

The amounts of DOC were influenced by mineral N fertilization (significant increase at maximum N dose). In addition, there was a significant interaction between previous crop and N fertilization, so that the combination of both factors led to a strong increase in DOC in 0-30 cm. The values were significantly higher after clover mulch than after field beans. This effect is associated with the fact that after clover mulch more plant biomass remains on the soil, which leads to more DOC through microbial degradation. Water-soluble C-compounds that result from this are mainly water-soluble carbohydrates (mono- and disaccharides), decomposition products of fibrous materials, but also polyphenols (phenolic acids, flavonoids) and other secondary plant compounds (coumarins). In addition, soluble components of the humus fraction (humic and fulvic acids) could also have contributed to the DOC through their release. The amount of these compounds is apparently enhanced by the N fertilization applied to the subsequent crops.

In contrast to the TOC, the DOC values in the 30-60 cm layer were only slightly lower than the values in topsoil. However, the pre-crop effects in the topsoil due to the test factors could no longer be detected.

C/N ratio of the soil

The C/N ratio is a good indicator of the degree of decomposition and quality of the organic substances held in the soil. Buyanovsky et al. (1994) suggested that a lower C/N ratio in soil is indicative of organic substances that is highly processed and persistent and that organic matter with higher C/N is in the beginning phase of decomposition (Oades et al., 1987).

In the current investigation the C/N ratio ranged from 8.7 to 10.2 (in the topsoil 0-30 cm). The same level was found in arable soils by Han et al. (2017). However, within this variation,

no firm evidence could be stated that the C/N ratio changed because of different previous crops/soil uses. Although significant variations were found in two out of a total of seven studies, no plausible and directional changes could be observed. Thus, the C/N ratios under fallow were identical to the values under clover mulch. For this reason, it must be concluded that, due to these investigations, no lasting changes in the C/N ratio of the soil have taken place after almost four decades of carrying out experiments.

In contrast, changes of C/N were observed in other trials. Thus, Körschens et al. (2013) explained that increasing the percentage of organic fertilizers for the total C and N input will have adverse effects on C and N balances. It was stated that C/N ratio is a critical driver in organic matter decomposition regulated by soil microorganisms. The residues with higher C/N ratio led the higher carbon and nitrogen immobilization as for cereal straw (comparable with wheat, rye, barley and oat in our LTE), while legumes increase the N mineralization, which are famous for their narrower C/N ratio (Moritsuka et al., 2004). This tendency accelerates the decomposition of legume crop residues compared to cereal straw.

C:N ratio were also determined in another long-term experiment in Rothamsted located on arable lands characterized by clay soil conditions (Gregory et al., 2016). There different perennial grasses and a bare fallow land are included in crop rotation and no significant correlations were found between treatments and the C:N ratios of the soil profile. Thus, it was postulated that the uncertainties in C/N ratios attributed to the higher microbial oxidation of the crop residues over the long-term period as the soil is fulfilled with a proper aeration (Gregory et al., 2016).

Truong and Marschner (2018) explained the less frequent incorporating of crop residues mostly in the beginning of the experiment is contributed to nitrogen uptake as a source of energy by microorganisms. Thus, the most decomposed organic matter is that which was recently incorporated. Therefore, the frequent incorporation of crop residues not only meets the requirements of the soil microorganisms but also extend the decomposition of residues resulting in possible increase the C/N ratio of the soil. Also, the contribution of the root residues as well as vegetation types and the microbial communities are important factors in C/N regulation (Mooshammer et al., 2014).

In other long-term studies uncertainties were found between the nutrient inputs and C/N ratios or microbial biomass of the soil. For example, in the long-term experiment called DOK in Switzerland, no correlation was found between carbon and nitrogen inputs from crop residues or manure on one hand with C/N ratio and microbial biomass of the soil (Frossard et al., 2016). This finding is attributed to the soil aggregation existing in this experiment. In addition, they conclude that significant effect on nutrient microbial ratios only might occur over the 30 years and among extreme variant and control.

It is also essential to notify that the agricultural practices through residue management, symbiotic N₂ fixation and mineral NPK fertilizers as well as the climate conditions like precipitation and air/soil temperature play an important role in nutrient accumulation or losses in the soil profile (Kirkby et al., 2011; Xu et al., 2013).

5. 1. 4 CO₂ efflux (F_c) of the soil surface

C efflux per day and season

The carbon storage in the soil profile has an immense influence on global climate change. The global C efflux have been reported about ten times higher than the CO₂ emissions derived from human activities (Bond-Lambery and Thomson, 2010; Davidson, 2020). It is necessary to note that the amount of organic C accumulation in the soil from crop residues is not identical to the soil respiration derived from the feeding of microorganisms in the soil. Various soil management practices (such as changes of land use and tillage methods) can influence the C content of the soil (Lal, 2014). In addition, also climate may influence the respiration rate of the microbes that feed on soil carbon, and hence the CO₂ efflux of the soil (Davidson and Janssens, 2006). Therefore, acceleration of the C efflux of the soil leads increasing global warming issue (Davidson, 2020).

It's interesting to know the amount of CO₂ efflux in arable land, because there is no clear investigation of this kind of C losses in arable soils used by crop rotational farming. Therefore, we tried to figure out the long-term effect of cultivating systems on C losses, which can't be avoided but that are a part of that carbon going into the atmosphere and contributes to climate change.

In the own study in the first campaign (in 2018/19), the first year after cultivation of preceding crops we observed C losses of 3.6 g C m⁻² day⁻¹, which proposes the C loss of 109 g C month⁻¹ and subsequently, 1321 g C year⁻¹. In contrary in the second campaign the C losses were 2.2 g C m⁻² day⁻¹; 65 g C month⁻¹ and 787 g C year⁻¹.

Further on, it was found that the C efflux of the soil surface varied by the seasons. Thus, it was noticed that in winter the F_c values decreased first by cooling down of temperatures to 1.77 g CO₂-C m⁻² day⁻¹ and after a period of freezing an increasing trend in CO₂ efflux have been observed. Whereas, in the spring the F_c level of the soil increased to 4.25 g CO₂-C m⁻² day⁻¹ and in summer a further increase of F_c to the highest level of 5.18 g CO₂-C m⁻² day⁻¹ was found. In the second year the same tendency but with lower values was observed.

Seasonal variation of C efflux was also found by Jacinthe et al. (2002) indicated that the higher intensity of CO₂ emission occurred in the late winter (mean: 2.79 g CO₂-C m⁻² per day) and summer seasons (2.45 g CO₂-C m⁻² per day) and lowest in the autumn (1.34 g CO₂-C m⁻² per day). The application of straw mulch caused less variation in F_c compared to the control. However, total C losses were greater in the control (due to lack of C supply) than in the mulch variants (Jacinthe et al., 2002).

A similar study was done in Giessen based on the Atmospheric CO₂ Enrichment Experiment called Gi-FACE running on grassland. In this experiment an increasing trend towards higher soil respiration during the vegetation period in spring and summer was observed (Keidel et al., 2015) that was found also in the own study. In addition, no positive impact of the CO₂ enriched variants (eCO₂) on CO₂ efflux of the soil was determined (Keidel et al., 2015).

Climate effects on CO₂ efflux of the soil

Enhancement of soil organic carbon (SOC) stocks through mulching has been proposed, and although this practice can alter several soil properties, its impact on the temporal variability of carbon dioxide (CO₂) emission from soils has not been widely investigated. In the own study was found that there is a close correlation among air ($r(34) = 0.76^{***}$) and soil ($r(34) = 0.72^{***}$) temperature on one hand and CO₂ efflux of the soil on the other hand. But near surface CO₂ efflux was not affected by soil moisture ($r(34) = 0.21$ ns). The same result was described by Duiker and Lal (2000). The seasonal variation in CO₂ fluxes is noteworthy and reflects climatic control (esp. by air and soil temperature) on CO₂ production and emission, which is probably modulated by soil cover or further management practises.

In the own study it was noticed that by increasing the soil/air temperature in the summer also CO₂ emission increased and reached to its maximum level. Also, a lower trend but significantly higher values were observed in spring and autumn compared to winter. The seasonal effect makes clear that a change in the seasons will have an impact on C efflux. So, when winter becomes shorter and warmer, the amount of C efflux increases. Our study clearly indicated that the temperatures exerted significant impacts on seasonal as well as annual soil CO₂ efflux responses for different land use types despite the relatively weak role of moisture in controlling CO₂ efflux.

Further on, it was found that after a period of drought stress in summer (July – August) the evidence of precipitation (86 mm) has induced the soil respiration. This effect was also declared by Fierer et al. (2005) as well as by several previous studies (Raich and Schlesinger, 1992; Raich and Tufekcioglu, 2000). In accordance with our result other studies also declared that the intensity of CO₂ emission is higher in the summer season (2.45 g CO₂ m⁻² per day) and lower in the autumn (1.34 CO₂ m⁻² per day) (Jacinthe et al., 2002). In another study, Franzluebbers et al. (1995) indicated that the environmental factors such as soil moisture and temperature as well as seasonal variations have a direct impact on soil CO₂ emission and this effect differs depending on crop type.

In addition, we also observed a weak increasing trend in CO₂ efflux of the soil in the winter compared to autumn. It is hypothesised that this increase may occur by frost-killed soil microorganisms and the subsequent CO₂ release, which was also ascribed by Skogland et al. (1988). A further reason of higher effluxes in winter could be the presence of soluble organic C, which is released from disrupted soil aggregates (Edwards and Cresser, 1992).

On the other hand, soil moisture is a crucial environmental factor aside temperature is driving the soil respiration. Soil moisture can alter soil CO₂ efflux by changing substrate availability and by altering the composition and activity of decomposer microbes (Williams, 2007). Thus, limiting soil moisture can suppress microbial activity independent of soil temperature and thereby decrease the responses of soil CO₂ efflux to the temperature (Davidson and Janssens, 2006). However, a relatively poor relationship between both factors is generally found, which indicates that soil moisture tends to have an indirect effect on soil CO₂ efflux. Therefore, it is difficult to ascertain the responses of soil CO₂ efflux to soil moisture.

Previous studies noted that soil moisture does not always statistically correlate with CO₂ efflux, although soil moisture is considered one of the important variables that help to drive soil C emission (Davidson et al., 1998; Saiz et al., 2007; Kechavarzi et al., 2010; Suseela et al., 2012).

Effects by the cropping system (or land use) and NPK fertilization

Within the 10th crop rotation of the current LTE “BNF” investigated here and among sixteen individual observations during the growing season 2018/19, we found a large variation between different preceding crops and mineral fertilization on CO₂ efflux of soil. Although the pre-crop effect on CO₂ efflux was not stable during the growing season, we found significant indications of higher soil respiration in clover (54 µg C m⁻² s⁻¹) and oat (62 µg C m⁻² s⁻¹) compared to fallow (45 µg C m⁻² s⁻¹) in average in 2019 in the first year after previous crops. This result could be interpreted as a sustainable change in SOC or DOC levels in the soil provided by different preceding crops.

In contrary, in the growing season 2020 no effects of preceding crops were observed. This suggests major variations in efflux readings that have occurred in that year. Regarding this, Jacinthe et al. (2002) showed that soil management with organic mulching (straw mulch) had a positive impact on CO₂ fluxes depending on seasonal variations. Therefore, the soil temperature has a crucial role on mineralization of organic matter such as mulch on the soil surface and subsequently the regulation of CO₂ emission which has also been shown in the studies of other authors (Raich and Schlesinger, 1992; Franzluebbers et al., 1995; Fortin et al., 1996; Mielnick and Dugas, 2000). This trend was also noted in many previous studies performed in a variety of ecosystems (Tang et al., 2009; Polley et al., 2010).

The decomposition of organic residues in soils lead to mineralization of different C fractions by microorganisms and releasing the organic compounds to increase the SOC pools in the soil. Based on other studies it can be stated that microclimate (e. g. soil humidity and temperature; Schomberg et al., 1994), the quality of crop residues (Tian et al., 1993; Trinsoutrot et al., 2000), and placement (Trinsoutrot et al., 2000) may also affect residue decomposition in soils.

Regarding NPK fertilization the own result has shown no effect of fertilization on CO₂ efflux during the first measurement period (campaign 2018/19). In average the Fc within NPK fertilization was 4.07 µmol m⁻² s⁻¹ compared to 4.53 µmol m⁻² s⁻¹ in the control. Furthermore, during the second measurement period (campaign 2020/21) the effect of NPK fertilization on CO₂ efflux was not clear. Thus, the assumption that increased N fertilization leads to more C-input, more microbial activity and higher C-respiration (Fc) was not confirmed in our own study.

Regarding N fertilisation similar result has found in other studies. For example, Jacinthe et al. (2002) also found no influence of mineral N fertilization on CO₂ fluxes. They have stated that fertilization deficiency effect has indicated that litter degradation was not dependable on N deficiency and the soil was able to release the mineral N gradually to sustain the function of the soil microorganisms. Also, in further studies has been reported that N fertilization has had restricted or no existing reaction on degradation of organic substances (Corbeels et al., 2000), forest litter (Castro et al., 1994) and SOC in arable soils (Green et al., 1995).

Methodological aspects

Utilizing the soil chamber method is more economic than soil CO₂ gradient method which is used in studies in particular on larger experimental sites and with multipoint measurements. On the other hand, the measurement with a portable device (chamber method) needs to be carried out by the operator, which is time consuming. In our case we carried out the measurement with only one portable device (Li-870 with smart chamber). The duration of measurement for each collar was 180 seconds, a time expenditure, which was applied in the current LTE in 120 rings distributed among selective treatments of 80 plots. The regular measurement starts from morning and ends up in the evening and includes more than 9 hours measurement. Since the CO₂ efflux rate is dependable to the air/soil temperature a day-time period can be characterized as an important variable to raise the variation through the measurement.

In this regard Pingintha et al. (2010) showed that the CO₂ efflux peaked at around 18:00 hour, 2 – 4 hours later than the peak of soil temperature in 0.02 m depth. Between 14:00 and 16:00 hours when soil temperature at the 0.02 m depth showed the highest value within a day, the CO₂ concentration at the 0.02 m depth had a minimum value.

Further on, the reasons for decreased CO₂ concentration in the topsoil layer (0.02 m depth) at high soil temperature have been discussed. Tang et al. (2003) attributed this result to the CO₂ production rate and diffusivity at that layer. CO₂ production rates are sensitive to soil temperature, but any additional increase in temperature may reduce the temperature sensitivity and CO₂ production rates (Lloyd and Taylor, 1994; Xu and Qi, 2001).

Another reason for the decreased CO₂ concentration at high temperature is the transport of CO₂. The high transport rate of CO₂ may prevent CO₂ from building-up in the top layer during early afternoon because CO₂ diffusivity increases with temperature (Tang et al., 2003). In addition to soil biological and physical factors, the surface wind may also affect CO₂ concentration (Pingintha et al., 2010).

Due to the variation in soil texture and water content there is an influence of these factors on calculations of the soil CO₂ diffusion coefficient. Therefore, it is presumed that the availability of more accurate soil temperature and water content sensors installed adjacent to the soil CO₂ concentration probe array can improve the accuracy of the measurements.

Furthermore, CO₂ efflux investigation requires highly accurate measurements of small flux rates. Therefore, it is critical to put the smart chamber appropriately on the collar to seal the defusing system. During measurements any plants, plant leaves or other plant residues (under the chamber) should be avoided because it may cause a leak in the system and can increase the CO₂ efflux value unexpectedly.

Further on, it was found that under drought stress soil cracks can also interrupt the measurement and induce varying results. Thus, it is recommended after a period of time to fix the collars and recover the cracks if exist. If all these circumstances are met the efflux measurements can be suitably performed.

Overall, it can be presumed that the efflux measurements with the method used here can provide reliable results if the investigations are carried out with great frequency and accuracy. The method is flexible and can be used on small plots with varying test factors. A disadvantage is the high manual effort required for measurement and the dependence of the measurement results on soil and environmental conditions. According to the hypotheses put forward, it can be stated that a seasonal and temperature-dependent fluctuation of the efflux rates was confirmed. However, the suspected influence of the previous crop and the NPK fertilization could not be proven, probably caused by high variation of the data.

5. 1. 5 Cation exchange capacity (CEC)

The cation exchange capacity (CEC) is a measure of the exchangeable cations of the mineral and organic colloids in the soil and thus describes the characteristics of the soil to store nutrients (cations) and release them again (to plants). Soils with higher CEC have a higher capacity to retain reasonable quantities of cations especially those as Ca^{2+} , Mg^{2+} , NH_4^+ , Na^+ and K^+ that are important for plant nutrition and plant growth (Ross and Ketterings, 2011). In addition, soils with a greater CEC may not necessarily be more fertile because they can also be occupied by acid cations such as hydrogen (H^+), aluminium (Al^{3+}) and iron Fe^{3+} (Ketterings et al., 2006; Weil and Brady, 2016). Further on, many heavy metals like Cadmium (Cd^{3+}), Nickel (Ni^{2+}), Copper (Cu^{2+}), Zinc (Zn^{2+}) or Chrome ($\text{Cr}^{3+}/\text{Cr}^{6+}$) are also cations and can thus be bound to soil colloids and absorb by plant roots (Sonon et al., 2017).

However, CEC values in combination with other soil parameters provide knowledge of soil quality and its productivity, pH value, soil aggregation stability and soil's interaction with organic and mineral fertilizers (Hazleton and Murphy, 2007). It is acknowledged that CEC of a soil is strongly associated with the clay and organic colloid fractions. For this reason, an increase in clay content (through amelioration) or humus content (through the addition of organic matter) can lead to an increase in CEC due to the increase in mineral or organic soil colloids (Caravaca et al., 1999; Radulov et al., 2011).

The increment in application of organic residues will likely increase the CEC of the soil over time (Ross and Ketterings, 2011). Nevertheless, the CEC can decrease with time as well, through e. g. natural or fertilizer-induced acidification and organic matter decomposition (Ross and Ketterings, 2011). Thus, it was noticed that long-term use of anhydrous ammonia and urea led to soil acidification in silty loam soils (Bouman et al., 1995; Thomas et al., 2007).

In the own investigation, it was evaluated whether the regular application of green mulch leads to improvements in CEC after a longer period of time, while fallow land leads to a decrease in CEC. However, this assumption could not be confirmed in the LTE "BNF" Giessen because the different preceding crops did not affect the CEC of the soil. The CEC values of the upper soil (0-30 cm) investigated in March 2019 were statistically identical between all preceding crops including fallow land. This result is surprising, as several other relevant measurements have noticed that C as well as N accumulation and thus the humus content in the soil has increased. Thus, it could be shown in the own study that the values for Ct (only 2019 and 2020), DOC and TOC as well as for Nt and DON have increased in the topsoil in the

variant of clover mulch. Therefore, we would have expected that with the significant increase in organic C and N in the soil, the proportion of organic colloids in the form of humic acids would also increase, which could subsequently increase the CEC value but that was obviously not the case.

The causes for this seemingly contradictory finding cannot be clearly explained, especially as there is a lot of evidence from research that the addition of organic matter to the soil over a period of more than 30 years can change the CEC. Thus, it has been observed in other studies that CEC is closely associated with the content of organic matter in the soil. It increases slowly with the time by the accumulation of organic residues, mostly in the topsoil and further also at deeper layer (Bot and Benites, 2005). Also, further studies noticed that soil organic matter has a positive effect on CEC over a long time (Czarnecki and Düring, 2015; Schjonning et al., 1994).

However, it must be considered that in other experiments, different conditions existed compared to the own LTE "BNF" Giessen. For example, Schjonning et al. (1994) examined a longer LTE of 90 years, which was located on a sandy loam soil. It could be that the period of humus accumulation in the own LTE "BNF" Giessen was not yet sufficient to increase the CEC. In addition, the soil type may also play a role. For example, a higher content of clay or organic substances in the soil may cause a higher CEC value and higher water hold capacity than sandy soil (Ketterings et al., 2007; Sonon et al., 2017). Thus, Sonon et al. (2017) cite a CEC of 1 to 5 meq/100 g in sandy soil compared to > 30 meq/100 g in clay soil, and levels of 200-400 meq/100 g in the soil with higher organic matter.

Further on, sandy soils are more susceptible to K and Mg (and other cation) deficiencies. In addition, also the soil pH may decrease faster by the time, where there is lower CEC in the soil (Ketterings et al., 2007). Therefore, sandy soils need to be limed frequently than clay soils, but they require less lime compared with clay soils to improve the pH to appropriate levels (Ketterings et al., 2007). It is also possible that CEC effects are faster and more pronounced on a sandy soil than on a clay soil, as is the case in the LTE "BNF" Giessen. For example, Weil and Brady (2016) studied sandy soils, which contained low levels of mineral and organic colloids and therefore had a lower CEC than clay and loam soils. They pointed out that the very high CEC is associated with humus compared to those exhibited by the inorganic clays, especially kaolinite, Fe and Al oxides.

In contrary to the preceding crops, NPK fertilization has significantly increased the CEC values of the topsoil compared to the control. Thus, maximum N fertilization to the crops has improved CEC value of the topsoil via increasing the biomass production which in parallel have caused an increase in root mass and in plant residues (e. g. stubbles and leaves) in the soil.

This result is in agree with the results of Brar et al. (2015) who indicated that CEC can be higher where mineral fertilizer (NPK 150%) is added and lower where N 100% or FYM manure plus NPK fertilization was applied. Also, Shukla et al. (2020) have shown that higher levels of NPK fertilizers released a higher number of exchangeable cations on clay micelle. Further on, in a study from Cakmak et al. (2010) was found that 40 years of application of P fertilizers significantly decreased pH and increased CEC of the soil.

Also, Czarnecki and Düring (2015) have investigated the relationship between NPK fertilisation and CEC based on a long-term study. They observed that nitrogen application as calcium ammonium nitrate-based fertilizer only or accompanying with NPK complex fertilizer can increase the CEC of the soil. This finding is in adherence to the own result and can be answered by soil colloid retention of applied Ca^{2+} , NH_4^+ , and K^+ ions (Radulov et al., 2011) and by the increased SOC content, which is reasonably an essential parameter affecting soil CEC (Rashidi and Seilsepour, 2008). In contrary, Thomas et al. (2007) have noted that there are no significant effects of stubble retention or N fertilizer application on CEC in the 0-10 cm depth.

Clay and silt content of the soil are very essential in the stabilization of organic compounds (Bationo and Buerkert, 2001) and CEC is depended directly more on SOC than on soil clay content (Bationo et al., 2007). Thus, it was shown that a difference of 1 g kg^{-1} SOC may result in $0.25 \text{ cmol kg}^{-1}$ for soil CEC (Ridder and Keulen, 1990).

Other LTEs have shown that not only different mineral fertilizers like N, P or combination of NP and NPK have an influence on the CEC, but also further factors like the amount of mineral fertilizer supplied, climate conditions and soil properties like SOC (as explained above) and pH should be taken into account (Czarnecki and Düring, 2015; Solly et al., 2020; Tirol-Padre et al., 2007).

Overall, it can be stated that in our own investigations only the N fertilization, but not the leguminous previous crops (incl. clover mulch) contributed to the increase in CEC. Possibly the trial duration with a total of nine preceding crops (in nine rotations) was still too short to supply sufficient biomass leading to more organic colloids (humic acids) in the soil. Therefore, repeating these measurements after a few more rotations is recommended.

5. 1. 6 Soil microbial biomass carbon (SMB C)

SMB C is considered as the living part of organic matter that contribute effectively in decomposition and mineralization of plant and animal residues in the soil (Marinari et al., 2006). Thus, they are more sensitive and react much faster to the soil conditions than other factors like organic matter (Brookes et al., 2008; Brookes, 2001). SMB C is considered to be a determinant of land use management and changes in SMB C are directly associated with important functions, such as organic matter decomposition and nutrient cycling (Nunes et al., 2012; Vinhal-Freitas et al., 2017).

In our own study, SMB C was determined a total of five times (2016, 2018, 2019, 4/2020 and 10/2020). Significant differences were only found in two of these studies. The clearest results were from 2019, where clover mulch together with oats achieved the highest SMB C values, followed by field bean and maize. Whereas the values were lowest on the fallow land plots. In addition, there was also a clear N effect on SMB C values this year. These results from 2019 confirm the hypothesis that legumes and mineral N fertilization can increase SMB C in the topsoil. However, this statement is not in conformity with the results from the other years, where either no effects were obtained (2016, 4/2020, 10/2020) or a somewhat contradictory result was obtained (2018). In 2018, there were significant variations between

the treatments, but the SMB C values of clover mulch and fallow were the highest and identical to each other.

Based on these results, it is suspected that the SMB C values depend on how long the period was between the previous crops and the collection of the soil samples. In 2019 (one year after the preceding crops), the positive effect of the preceding crops was obviously still good to demonstrate. In contrary, no certain effects were observed in 2016 (two years after the previous crops) and in 2020 (also two years after the previous crops). This finding suggests that the microbial biomass of the soil is not yet sustainably changed by the cultivation of legumes, which took place every four years.

In some studies, the potential of crop residues of preceding crops was investigated (Francis and Clegg, 1990). It is stated that the plant residues may improve root growth of subsequent crops, which afterwards promise more stability of microbial biomass in the cropping system.

In the own study in 2019, same SMB C compared to clover mulch was observed in oat. This higher SMB C of oat could be related to the quality of the organic matter resulting into easier decomposability of the plant residues of oat. In convergence to this finding García-Orenes et al. (2013) reported treating the soil with oat straw residues is a sustainable management practices to increase the soil microbial biomass and their activity. They demonstrated that the soil managed by oat straw contain higher fatty acid concentrations, which indicating higher versicular-arbuscular mycorrhiza and bacterial activity and thus higher SMB C content. This hypothesis is also supported by a former investigation from Frostegard et al. (1993).

Other study showed that the increased root growth would not only provide more root mass for decomposition but would also provide root exudates and a favourable environment for the microbes and its activity during the growing season (Haichar et al., 2008). Further on, crop diversity may also reduce soil borne diseases and thus increasing the SMB C of the soil. Also, crop residue management increases the SMB C by improving soil biological properties (Francis and Clegg, 1990). Moreover, microbial growth (Blagodatskaya et al., 2009) and its activity (Nannipieri et al., 2012) can be encouraged the roots to release the labile compounds and enzymes.

Williamson and Johnson (1994) indicated that adding organic materials or maintaining a soil-covered surface is a suitable management methods of soil regeneration to conserve soil microbial biomass and to improve soil properties. In another study it was noticed that the addition of a green manure to wheat-based systems in the semi-arid region over a 30-year period led to higher microbial biomass caused by increasing soil enzyme (e. g. urease, phosphatase, and dehydrogenase) activities (Bolton et al., 1985). In disturbed ecosystems, the high respiration rate per unit of microbial biomass presence intense competition for small amount of available C, which induces microorganisms to take more C as a source of energy for their maintenance and increasing population (Moscatelli et al., 2007).

The vegetation cover has also fundamental effects on soil properties (Rutigliano et al., 2004), because it contributes to increase the organic matter to the soil and protects the soil

from erosion. Generally, a reduction in green surface results in decreasing organic matter to the soil ecosystem and may emerge as a C deficit and low microbial biomass potential (Powlson et al., 1987). The mechanism responsible for the recovery of soil microbial biomass could be that the utilization of organic and green manure stimulates the growth of soil microbial biomass by increasing the input of plant residues and plant roots (Zhang and Fang, 2007). In contrary, the absence of vegetation cover exposes the soil microbial biomass to the effects of rain and to the high temperatures found mainly in the surface layer of soil (Nunes et al., 2012).

A further factor is the plant diversity which mainly influence the soil microbial activity by altering the rhizo-deposits and litter (Wang et al., 2017) and may be modified by species characteristics, biomass production and soil environments. In contrast, other studies (Johnson et al., 2008; Rottstock et al., 2014) showed negative or no correlation between soil microbial biomass, activity and diversity on one hand and increasing plant diversity on the other hand.

In the own study, mostly a higher C and N content of the soil was observed in cultivation system with 25% legumes (clover mulch) and in treatment with 100% NPK, what might have contributed to the maintenance and the growth of soil microbial biomass, as reported also by Araujo et al. (2010). Further on, Hao et al. (2008) indicated that the application of NPK fertilizer had little effect on SMB C in a long-term experiment. In their investigation was observed that the application of mineral NPK fertilizer in combination with manure has significantly increased SMB C contents by 21, 51 and 65% in NPK plus medium rate manure and by 26, 58 and 79% in the NPK plus high-rate manure treatment, respectively (Hao et al., 2008).

Also, other studies have clearly indicated that different organic amendments (straw and other crop residues, FYM and compost) and diversified crop rotations, particularly those, which include grasses or grass-legume leys, beneficially affect different soil processes and properties, including accumulation of SOC, SMB C and stimulation of soil biological activities (Scherer et al., 2011; Scotti et al., 2015). This effect is caused by increasing acid phosphatase and dehydrogenase activity as by transforming larger amount of fresh organic matter and the stored SOC (Martyniuk et al., 2019).

In another long-term study from Hopkins and Shiel (1996) was noticed that the microbial biomass was significantly higher in soils managed by farmyard manure in combination with NPK fertilizer than those only managed by mineral NPK fertilization. Moreover, Ocio et al. (1991) have shown the application of straw amendments to the field significantly accelerate the soil microbial biomass. Both studies thus underline the positive effect of manure and straw mulch on the microbial biomass in the soil.

Eekeren et al. (2008) noted that the reducing trend in microbial biomass in agricultural soils, which emerged as about 34% reduction in bacterial biomass or about 21% decrease in fungi in the grasslands. Further on, the researchers observed also that the bacterial biomass was 50% higher in permanent grassland compared to permanent arable land. Also, it was shown that in croplands the SMB C level was reduced by 30% to 80% compared to fallow land (Wolińska et al., 2014), which indicate a negative influence of farming treatments on soil biological parameters. Due to this observation, croplands may contain less stable microbial communities.

In a soil quality monitoring network in Germany, bacterial biomass was also 50-100% higher in grassland compared to arable land (Bloem et al., 2006). Further on, Fromm et al. (1993) have shown a robust correlation among soil carbon accumulation (which is higher under grassland) and microbial biomass. Furthermore, it was found, that grassland soils are characterized by lower bacterial growth rate compared to arable land (Bloem et al., 2006; Garbeva et al., 2006). Thus, agricultural soils may support fast-growing colonizers because organic matter inputs are very seasonal (Bloem et al., 2006).

Generally, cultivation is supposed to modify the quantity of soil organic matter resulting in reducing nutrient availability (Schlesinger and Bernhardt, 2013). It was observed that crop diversity in land use management changing the soil microorganisms and their communities (Rodrigues et al., 2013). Since microbial communities of the soil make a fundamental contribution to soil functions; their richness, and composition are extremely dependable to the land use strategy and management practices (Trivedi et al., 2016). Changing land use, rotating different crop species, and the application of mineral fertilizer are common soil practices that can considerably affect the soil organic matter content (Murillo, 2001; Paustian et al., 1997). Conversely, Martyniuk et al. (2019) indicated that the application of mineral N fertilizers in different rates have no significant influence on SMB C when no manure was applied to the soil in a rotation (grain maize, winter wheat plus mustard green manure, spring barley and grass-clover hay), therefore, among the other variants the increasing rate of mineral N cause a decreasing trend in soil microbial biomass.

Also, there is a general conformity that increasing SOC sequestration caused by the application of organic and mineral N fertilization and appropriate soil management practices on croplands, which is very effective for the soil microbial activity and soil microorganisms mainly due to a greater recovery of crop residues to the soil (Acosta-Martinez et al., 2007; Martyniuk et al., 2019). However, there are also reports showing that mineral N fertilizers, particularly in higher rates, can reduce SMB C and soil enzymes activities by changing the soil pH and decreasing acid phosphatase and dehydrogenase activity (Ghimire et al., 2017; Liebig et al., 2002). Although the microbial biomass represents a limited fraction (typically 1–5%) of SOM (Sparling, 1997), it contributes crucially in mineralization of the nutrient and decomposition of the soil organic matter (Wu et al., 1993; Kibblewhite et al., 2008).

Therefore, it can be supposed that SMB C is a fairly simple parameter that can also be well determined in LTE and can be considered as a measure of soil health. There is much evidence that SMB C is improved by organic fertilization, plant residues, mulching practices and legumes. This effect was also partly demonstrated in the own LTE study, especially in the first year after cultivation of the preceding crops.

5. 1. 7 Mesofauna abundance and mesofauna feeding activity in the soil

Because of the great importance of the mesofauna for soil fertility and because it can be influenced by land use and its management, this biological parameter was also investigated in the LTE "BNF" Giessen by two methods, mesofauna abundance and feeding activity. The results that have been achieved showed, across all cultivation systems, the land use with a

proportion of 25% legumes (crimson clover) within the crop rotation has led the higher number of arthropods in the topsoil compared to fallow land or the land with a proportion of 100% cereals.

Furthermore, this effect is attributed to the plant biomass that remains on the soil during mulching or after harvesting. This is especially true for clover mulch, with all the above-ground biomass remaining on the soil. These plant remains provide a protective layer for the soil surface against water evaporation, which has prevented the soil from drying out. In addition, a mulch layer also causes a certain temperature regulation and a flattening of the temperature amplitudes at the soil surface. Both meteorological factors have probably promoted the mesofauna population (Kaneda et al., 2012; Nakamoto and Tsukamoto, 2006).

Plant residues (above ground and root mass) are left in the soil by all plants during growth and especially after harvest. This is also the case with cereals. However, in contrast to cereals legumes usually have a greater root depth and mass, which is beneficial for the soil fauna. In addition, legume biomass contains more nitrogen and a narrower C/N ratio, which improves its degradation and metabolism by soil animals. This relationship is confirmed by Milcu et al. (2008), who noticed that soil fauna directly took advantage of nitrogen rich litter resources or root residues with higher nitrogen rich rhizobia (Milcu et al., 2008). Also, in the study from Fu et al. (2009) was found, that green manure (clover) practices or crop residues influence the soil fauna abundance in farming systems (Fu et al., 2009; Tremelling et al., 2003). Another explanation might be that the nutrient composition of organic residues among other species, for instance, legume mulch is more attractive to soil organisms (Birkhofer et al., 2011).

The second parameter determined in the own study was the feeding activity of the soil arthropods. The investigations of the own study performed in 2020 revealed, the feeding activity of the soil arthropods was significantly higher in the soil cultivated with 25% legume (clover mulch) than the land under continuous cereal cultivation (100% cereals) or 25% fallow lands in the rotation. This result attributes to the root as well as the potential of legumes in biomass production. In addition, the nutrient value of the organic matter effectively regulates and moderates the soil feeding activity (Henriksen and Breland, 1999). Further on was observed that high C/N ratio of cereal straw caused low decomposition rates (Martens, 2000; Sieling, 2019) and reduced the attractiveness or palatability of straw for soil invertebrates (Ruiz et al., 2008).

Accordingly, it was found that the soil fauna abundance differently reacts to the mineral NPK fertilization. Although the total number of arthropods was not affected by the main treatments (pre-crops and mineral NPK fertilization), but interestingly it was found that springtails significantly and positively responded to NPK fertilization. In contrary, no changes were found on mites, pauropoda or other communities which were identified. This result can be acknowledged by increasing plant growth (including underground biomass/roots) in NPK fertilizer treatments, which presumably caused an increase in microbial biomass, especially fungi, a major food resource of Collembola (Schütz et al., 2008).

These effects raise the possibility of toxicity due to the increase of heavy metals in soil as a consequence of long-term fertilization. When the heavy metals bounded with the soil colloids,

the soil fertility significantly reduce or these heavy metals absorb by crops (De Vries et al., 2013; Kabata-Pendias, 2011). Heavy metals like cadmium (Cd), lead (Pb) and arsenic metalloid (As) have been found in P fertilizers and are considered the most important of health concern (Gupta et al., 2014). Moreover, it was pointed out that recycled P fertilizers were the main source of the heavy metals in arable lands (Weissengruber et al., 2018). In convergence, Cao et al. (2011) showed that the long-term continuous (11-year) application of organic and chemical P fertilizer treatments reduced the abundance and diversity of soil fauna.

Many laboratory efforts have been made to reveal the relationship between heavy metal and soil fauna. It is plausible that soil fauna can be restricted by heavy metal (Didden and Rombke, 2001; Herbert et al., 2004). Also, several studies showed that heavy metal in agricultural soils can decrease soil fauna (mites) abundance and richness (El-Sharabasy and Ibrahim, 2010; Migliorini et al., 2005). Further on, it is showed that Pb and Zn accumulation has negatively influenced the existence of Mesostigmata mites and decreased soil mesofauna species richness (Manu et al., 2019; Yu et al., 2021). The same trend has been found in collembolan by Santorufo et al. (2012).

Other studies have also shown that chemical fertilizers decreased the diversity of soil mites (Cao et al., 2011; Minor and Norton, 2004). For example, long-term studies carried out by Zheng et al. (2000 and 2002) on the impact of different chemical fertilizers on the soil mites community showed decrease in both abundance and diversity of mites after eleven years.

Kautz et al. (2006) found that the green manure and residue management increased the soil arthropods abundance, contrary to mineral nitrogen. They also highlighted that fertilizer application could not take enough time for a significant induced modification of the fauna composition. In spite of that, Cluzeau et al. (2012) explained that collembola abundance was increased by both organic fertilizers and mineral inputs, while mites responded more directional to the mixture of manure and mineral fertilization compared with the sole mineral application (Gruss et al., 2018).

On the other hand, climate condition like air/soil temperature and precipitation are essential to build up the soil microarthropod populations as the climate condition is a limiting factor for vegetation growth to provide an appropriate habitat for the soil microorganisms (Kardol et al., 2010). Thus, Kardol et al. (2011) have explained a positive correlation among the soil water content and the richness of the microarthropods. Moreover, the soil microarthropods also respond differently to the soil condition. For example, collembolan take advantage of the soil moisture to build up their communities rather than mites as no significant relationships were found for mites (Kardol et al., 2011).

In the own experiment, we evaluated the hypothesis whether mineral NPK fertilization or land use which includes legumes can improve the soil mesofauna abundance. In the current LTE "BNF" it was found that the soil fauna reacts differently to the environmental circumstances. The impacts of different cropping systems and mineral NPK fertilization could be partially confirmed. Thus, mites as well as collembola abundance were dependent on mineral N fertilization and those land uses which included legumes.

5. 2 Effect of preceding crops/rotational crop diversity and mineral NPK fertilization on plant parameters of the subsequent crops winter wheat, winter rye and summer barley

5. 2. 1 Plant growth parameters (vegetative tillers, spikes, plant length)

High yielding cereal production systems need to determine the optimum plant population development (number of stems per unit area including main tiller density), which is very essential in balancing the interactions between the three yield components including spike/inflorescence number, grains per spike, and thousand grain weight (Lu et al., 2016; Sieling et al., 2005). This also applies to the cereals winter wheat, winter rye and spring barley, which were examined in the current LTE "BNF".

Density of vegetative tillers

Tillering stage is a very important detective phase in cereal crops to promise yield and somehow indirectly the yield components. The number of tillers vary depending on the genetic characteristics of the plant species or the growth conditions (Bauer and Wiren, 2020; Diekmann and Fischbeck, 2005; Simmons et al., 1982). Thus, the tillering capacity is very important in cereal as the higher tillering rate often prompt to leave some insufficient filled grains, while the lower tiller densities do not provide the potential yield (Berry et al., 2003).

The own study revealed that in winter rye 2016 clover mulch significantly increased the density of vegetative tillers compared to other land uses (pre-crops). This trend was surprisingly significantly higher in crimson clover than in field bean, which is attributed to the quality and amount of the plant residues which remained on/in the soil. Contrary to clover mulch after harvesting field bean only straw residues remained on the ground, while crimson clover was incorporated in the soil as a green manure. In contrary, in summer barley 2017, no significant differences were observed between previous crops, but crimson clover again led to (not significantly) higher density of vegetative tillers. Thus, the N accumulation in the soil seems to have a positive effect on the formation of tillers even in the early stage of plant development.

Beside the pre-crop effect it was also observed that in both years 2016 and 2017, the vegetative tiller production significantly responded to the dosage of mineral N fertilization. This effect was expected due to the direct N-fertilizer effect on the growth of the cereal plants. Similarly, it was found that the number of tillers is strongly correlated to N fertilization (Bauer and von Wirén, 2020). In addition, in the management system mineral fertilizer and water use efficiency often determined by the number of tillers and the biomass ratio (Blankenau et al., 2002; Guo and Schnurbusch, 2015). However, tiller density often decreases by the crop development especially during the generative phase, which strongly associated with parameters like sowing density, cultivars, light quality and water supply (water capacity of the soil) (Alzueta et al., 2012; Evers et al., 2006). Further on, it is explained that some combinations of mineral N are more effective than the sole formulation as the formation of tillers mostly stimulate by the mixture of ammonium nitrate (Wang and Below, 1996; Tanaka and Nakano, 2019).

Density of spikes

In the own study was found that within the 9th crop rotation the density of spikes was modified by the land use (preceding crop). So, that through the four years of the current crop rotation the use of green manure (clover mulch) effectively increased the spike density of wheat as a first subsequent crop. However, this trend was reduced over the second and third year of the crop rotation system, which is probably attributed to lower N content in the soil. Also, within the 10th crop rotation, the same pattern was observed in the first year of crop rotation after previous crops. In winter rye, cultivated in the third year of the crop rotation, higher spike density was found in the variant of green manure across all previous crops. However, this effect was not strong enough as in the second year of the crop rotation. These variations among the previous crops can firstly be explained by the quality and quantity of the biomass (shoot/root) production and secondly to the decomposition rate of the organic material in the soil which is also approved by Costa et al. (2021); Evans et al. (2003) and N'Dayegamiye et al. (2015).

In the own study it was also found, that within both rotation cycles (from 2015 to 2020) any input of mineral N fertilization has stimulated the tiller and spikes formation. The higher NPK dosage (particularly N) led to higher spike formation within the first, second and third year after cultivation of previous crops. It should be considered that the rapidity of plant growth, the utilization of N fertilizer, and the accessibility of N after application are subjected to weather conditions (Sabbe & Batchelor, 1990). Further on, the sole incorporation of residue or the application of N fertilizer with crop residues have positive impacts on plant growth and production (N'Dayegamiye et al., 2015).

Plant height

The plant length (plant height) indicates the reaction of the plants to the growth factors like water, temperature, UV light and nutrient supply (esp. nitrogen). Furthermore, plant length is genetically determined and can also be influenced by inter- and intra-specific competition within plant populations (Barillot et al., 2014). In cereals, plant length is also modified by growth regulators applied by the farmer to stabilize the stalks and to prevent or minimize lodging of the plant stands (Peake et al., 2020). Growth regulators contribute to stalk shortening and stalk strengthening by inhibiting growth hormones (esp. auxins and gibberellic acids) which induce and promote the longitudinal growth of the internodes (Kupke et al., 2022; Marzec and Alqudah, 2018). Longer culms also have a competitive superiority to the neighbor culms and thus have advantages for ear formation.

In the own study it was found that biological fixed nitrogen and the mineral N inputs to the cropping system directly influenced the plant height of subsequent crops because nitrogen is a limiting factor of plant growth development and yield formation. This observation is in agreement with other studies from Sieling et al. (2006) and Sieling and Christen (2015) that reported that increased soil N amount, and the soil N mineralization potential may develop plant growth and support the formation of yield.

In the LTE studied here, NPK fertilization caused an increase in stem length in all trial years, as expected, which can be associated with the direct effect of the mineral N. Increasing the N dose from NPK 50% to NPK 100% led to a further increase in stalk length. This effect confirms the positive impact of N nutrition on length growth and biomass increase in cereals (Blumenthal et al., 2008; Guo et al., 2019).

Further on, it can be stated that plant height known as the most important phenotypic traits in cereals which attributes to the architecture of the plant, lodging resistance, and yield performance (Wang et al., 2017). Extending the stem elongation phase is an important strategy to increase spike dry weight at anthesis (Guo et al., 2018), which would guarantee an adequate supply of assimilate for grain filling after anthesis (Ghiglione et al., 2008).

Of greater interest than the N fertilizer effect is the influence of the previous crop. Here it was found that in two out of four trial years the legume preceding crops promoted the length growth of the cereal subsequent crops. In 2017, this effect was observed in spring barley, which was cultivated in the third year (2017) after the previous crops (2014). This result is associated with the fact that even in the third year, more nitrogen was available in the soil in the legume variants, which could be mobilized for plant growth. This indicates that N accumulation in the soil was sustainably increased in these variants over several years (Foyer et al., 2019; Murray et al., 2017).

5. 2. 2 Leaf chlorophyll (SPAD), Leaf area index (LAI) and vegetation indices (NDVI/NDRE)

Leaf chlorophyll (SPAD)

The chlorophyll present in the plant leaves is closely related to the nutritional condition of the plant. Efficient N utilization improve leaf area index, radiation use efficiency, photosynthesis rate, and optimizing wheat yield and the quality parameters (Abid et al., 2016). Nitrogen plays a key role for maintaining crop health and crop growth regulations. Since, there is a strong correlation between chlorophyll content and nitrogen availability (Vigneaua et al., 2011). For that reason, the chlorophyll values often used as a good indication for regulating the application of nitrogen fertilization.

The concentrations of photosynthetic pigments strongly associated with the plant photosynthesis potential of a plant and, therefore, is representative of the photosynthesis status of the plant (Blackburn and Steele, 1999). Among these pigments, chlorophyll A and chlorophyll B absorb the largest proportion of photosynthetically active radiation (PAR), which provides energy for the reaction of photosynthesis. The carotenoids protect the reaction centers from excess light and help to intercept PAR as auxiliary pigments of chlorophyll a (Mõttus et al., 2012). Therefore, these pigments play an important role in plant productivity depending to the environmental condition. As the plant confronted with the stress condition, it decrease the chlorophyll content more quickly than the other pigments. Therefore, reducing chlorophyll concentration known as a common indication of stress condition. Furthermore, chlorophyll content in the plant strongly associated with the nitrogen concentration (Bannari et al., 2006). The chlorophyll concentration in the leaves not only is a good indicator of the

nitrogen concentration, but also presenting the status of leaf senescence that can also rapidly be affected by environmental conditions (Neufeld et al., 2006). The highest value content of pigments in wheat green leaves was reported at the end of flowering phase (Tranavičiene et al., 2008).

In the own study it was found that within the 9th crop rotation of the current “BNF” field experiment the variants with legumes (crimson clover as well as field bean) significantly increased the SPAD value of winter wheat in 2015, in the first year after the previous crops. This trend was lower in winter rye 2016 and summer barley 2017 (the second and third year of crop rotation), which attributed to the reducing effect of biologically fixed nitrogen by legumes over the second and third year of the crop rotation. Within the 10th crop rotation only crimson clover led to increased SPAD values of winter wheat in 2019 in the first year of the rotation after previous crops.

Further on, it was found that every dosage of mineral N fertilization led to significant increase of the SPAD values in the first, second and third years of crop rotation. The same trend was found within the 10th crop rotation as well. This effect is attributed to the enhanced nitrogen concentration in the leaves caused by mineral N fertilization. This is an expected and well-known effect that nitrogen is a central element of the chemical structure of chlorophyll. Therefore, the lower chlorophyll and protein concentration as well as smaller leaves and lower dry matter and leaf area index in cereal are the consequences of the inadequate N application (Walsh et al., 2020).

The determination of the chlorophyll content (SPAD) is also used to draw conclusions about other plant parameters. Thus, Takebe et al. (2006) have found a close relationship among protein content and leaf chlorophyll content at the full heading stage in wheat grains at maturity. So that, with the SPAD value of 50 to 52 at full heading stage the application of 30 kg N ha⁻¹ is required to reach the potential yield. Further on, Singh et al. (2013) found significant relationships between grain yield of wheat and the SPAD reading of wheat leaves at the maximum tillering stage. So that at the maximum tillering stage by reducing the SPAD values of the leaves, the potential yield of wheat linearly responds to the application of mineral N fertilization. For example, by reading the SPAD values of 32.5 and 42.5 at the maximum tillering stage, the usage of 30 kg N ha⁻¹ can increase the wheat yield up to 1.0 t ha⁻¹, (Singh et al., 2013).

Leaf area index (LAI)

LAI is broadly defined as the amount of leaf area (m²) in a canopy per unit ground area (m²). It is used to determine the plant's dry matter production in the vegetative period. Therefore, LAI is an important parameter for canopy architecture and biomass determinations. Better understanding of the leaf orientation in plant canopies and monitoring of plant development and productivity, predicting grain yield, and optimization of crop management practices provided by LAI measurement (Jin et al., 2013; Wang et al., 2016). The leaf density and orientations have a crucial role in plant photosynthesis potential and therefore dry matter formation by the crop. Therefore, the higher leaf density and the duration of photosynthesis leaves promising the yield and dry matter accumulation (Zhao et al., 2005).

In the own study the treatment with clover mulch led to significantly increased LAI values of winter wheat and winter rye within 9th and 10th crop rotation, across all previous crops. In addition, any mineral N inputs also significantly increased the LAI values of the crops. Interestingly the synergistic effects of clover mulch and mineral N fertilization on LAI of winter rye 2016 during the milk development stage (DC 73) was noticeable. The clear effect of legumes to increase LAI values is attributed to their potential to provide N to the cultivation system which leads to higher levels of plant-available N in the soil. Further on, legumes can modify the decomposition rate of the organic materials compared to non-legume crops which could also have contributed to a better or faster N supply of the subsequent cereal crops. For instance, Shi (2013) has shown that decomposition of clover in the spring is much faster than soybean which has a longer decomposition period than clover.

The positive correlation between the plant-available N and the leaf area index has been proven in several studies. Thus, it was found that N nutritional status, leaf area, and leaf area duration affect both short-term growth status as well as the future productivity of the crops. Further on, Wang et al. (2017) showed that the LAI and LAD (leaf area duration) increased with the increase of N levels while shoot nitrogen concentration decreased with the N levels.

In addition, Singh and Agarwal (2001) have found that the application of mineral nitrogen up to 180 kg ha⁻¹ significantly enhanced the dry matter accumulation of wheat at all growth stages. Also, Grashoff and D'antuono (1997) noted that the nitrogen fertilization led to increased LAI values of barley and Amanullah et al. (2007) indicated that higher N level have increased the LAI of maize crops.

Normalized Vegetation Index (NDVI)

The NDVI is well correlated with leaf area index (LAI) and is more sensitive to changes in the crop canopy when the LAI is low (i. e. during the early stage), with the signal becoming saturated when the crop canopy closes (Marti et al., 2007). The accurate monitoring of the N concentration status in crops in the beginning of the growth stages and adjusting the application of mineral N fertilization to ensure the yield production and reducing the risk of yield losses is critical. Hence, remote sensing methods can be effectively used to tackle this issue (Benincasa et al., 2018).

Some studies showed that the NDVI had a strong relationship with grain yield in wheat (Magney et al., 2016; Cao et al., 2015). Further on, NDVI has been effectively utilized in cereals to estimate the reflection of vegetation parts compared to the soil, which is representative of the plant growth (Lopresti et al., 2015; Mekliche et al., 2015).

Also, using NDVI during the growing season provides the environmental stress conditions in wheat and generally cereal cultivations (Lopes and Reynolds, 2012) as well as the requirements for the application of nitrogen fertilizer (Duan et al., 2017). In addition, by NDVI values it is possible to monitor the nitrogen content and regulate nitrogen use efficiency of crops (Erdle et al., 2011; Foster et al., 2016). NDVI not only indicated the crop growing stage but also provides more details like the moisture and nitrogen content of the soil (Edwards et al., 2015). Further on, it must be considered that NDVI measurement is strongly associated with the leave density and orientation of the canopy as well as growth condition (e. g.

vegetation cover, biomass of the plant, cultivars or species and soil moisture) or changes in growing conditions, e. g. temperature and water regime (Crusiol et al., 2016; Forkel et al., 2013).

In the own study, the increasing trend of vegetation indices was found across all previous crops compared to fallow land in winter rye 2016 within the 9th crop rotation. The same trend was found in summer barley 2017. In contrary, within the 10th crop rotation clover mulch obtained the higher NDVI values compared to the other preceding crops. Furthermore, mineral fertilization at both levels (PK+50% N and PK+100% N) significantly increased NDVI values over the 9th and 10th crop rotation. This positive trend is attributed to the availability of mineral N and N mineralization of the crop residues of different pre-crops as well as by the release of biologically fixed N by legumes.

There are several publications which prove the influence of nitrogen and other factors on NDVI. For example, Walsh et al. (2020) showed that the NDVI values were significantly affected by N fertilization or irrigation management. They found that treatments with no N input or irrigation resulted in the lowest NDVI, whereas high N input and irrigation treatment led to the highest NDVI values. The robust relationship among grain yield and NDVI supported the use of NDVI value as a reliable parameter for prediction of crop yield in cereal and N use efficiency (Raun et al., 2001).

Further on, it was shown by Benincasa et al. (2018) that higher LAI value also correlated with the NDVI parameter as increasing the leaf area is the result of extending more cover on the soil and reducing the ratio of the bare soil. Thus, NDVI provides the ability of screening crop N deficiency as well as other unfavorable environmental conditions, where the N requirement of the crop is promised. This is attributed to the fact that the precision and sensitivity of this measurements lies in expansion of the crop canopy on the soil and the quality of reflected light (red and infrared) from the vegetation surface (Ren et al., 2008).

5. 2. 3 Biomass yield, grain yield and harvest index

Crop biomass yield is the most important plant indicator of crop response to changes in soil properties. This crop response to soil properties can be modified by agronomic measures and climatic factors, especially water supply, depending on the genetic background of the respective plants (variety). The aim of the investigation carried out in the LTE "Biological N Fixation" was to clarify whether the different previous crops only had a short-term effect (in the first subsequent crop) or a long-term effect (in the second and third subsequent crops). The explanation to this question is of great importance for the evaluation of the sustainability of the preceding crops (or crop rotations) investigated here.

The research carried out has shown that within both crop rotations (9th and 10th crop rotation) the biomass yields (grain and straw yields) of subsequent crops were significantly increased by the land use which includes green mulch (crimson clover) in the first year of the crop rotation. However, this effect was clearer and more sustained in the 9th rotation (2014 – 2017) than in the 10th rotation (2018-2020). Thus, a clear increase in biomass yields after clover-mulch was observed in all three years during the study period 2015 to 2017. It is

noteworthy that the clover-mulch effect was also present in the second year (2016) for winter rye and especially in the third year (2017) for spring barley. The response of summer barley (three years after the preceding crops) shows that clover-mulch must have led to a lasting change in soil properties resulting in a clear biomass yield reaction of summer barley grown at the end of this rotation.

The afore-mentioned relationships apply not only to the biomass yield, but also to the two biomass fractions, grain and straw yield. Thus, it was noticed that the grain and straw yields of subsequent crops followed the same patterns as biomass yields within both crop rotations. Clover mulch outperformed the grain yield of winter wheat in the first year of both rotations (in 2015 and in 2019) compared to the land use with 100% cereal cultivation. Within the 9th crop rotation, interestingly the lasting effect of clover mulch led also to increase the grain yield of winter rye (2016) and spring barley (2017). Thus, it is also evident from the grain and straw yields that clover mulch has led to a significant change/improvement in soil properties over the years, enabling these yield effects.

The positive effects of clover mulch and partially also fava bean on the yields of subsequent crops in the entire crop rotation can be due to various reasons. One reason could be that clover mulch can sustain beneficial soil organisms like soil microbes and soil fauna communities by covering the soil with plant residues as well as by the quality of those plant residues (Marini et al., 2020; Tiemann et al., 2015). It is assumed that covering the soil with mulch layer may reduce water evaporation (Hartwig and Ammon, 2002) and thus preserving soil moisture. Further on, reducing the direct effect of UV light and wind on the soil surface covered by mulch can also be assumed (Klocke et al., 2009; Mitchell et al., 2012). Thus, in the study of Klocke et al. (2009) was found that the mulch cover of wheat stubbles and maize straw residues has reduced the soil surface evaporation by 50% to 65% compared to fallow with no surface coverage. These aspects can have a positive effect on microorganisms and the mesofauna. This relationship is supported by the results conducted by Ranaivoson et al. (2017). They explained that by adding 10 t ha⁻¹ crop residues from the legume crop *Stylosanthes guianensis* to the cropping system, the soil meso- and micro-fauna abundance has increased by 45% compared to bare soil.

A further aspect to ascertain the soil properties under clover mulch and fava bean is the positive impact on SOC and humus content. In the current study it has been shown that both legumes (crimson clover and field bean) have improved the SOC of the soil (see chapter 5. 1. 3) which is explained by the supply of aboveground and root biomass.

The plant biomass residues left by the respective previous crops which was determined in 2018 amounted to 64 dt ha⁻¹ by clover (grain + straw), 12 dt ha⁻¹ by field bean (straw), 62 dt ha⁻¹ by oat (straw) and 78 dt ha⁻¹ by maize (straw), respectively (table A 8). These data do not reflect the higher biomass input from the legumes, which could be due to the fact that the root biomass was not taken into account.

Yang and Kay (2001) explained that the amount of crop residues in different crop rotations consist of alfalfa, corn, soybean, barley, winter wheat and red clover, which were incorporated to the soil is very important to enhance SOC content. They found that soybean and corn

provide less residues compared to other species when they contribute to the cropping system. However, most of the studies do not determine the root mass, not even in the LTE's own measurements, which were carried out in 2018. Therefore, the root mass must also be taken into account when assessing the supply of biomass. In this regard, it can be presumed that in crimson clover and field bean a significant part of the root biomass is added.

The importance of crop residues of legumes and their incorporation into the soil to increase SOC is also confirmed by Diacono and Montemurro (2010) and Sanden et al. (2018). Further on, it is shown that root residues are very important to increase the SOC in the long-term legume crop rotation and the root residues have higher decomposing characteristics compared to the above-ground residues (Francaviglia et al., 2019; Rasse et al., 2005).

A further aspect could be that legume crops may reduce the CO₂ emission compared to other crops. This phenomenon is explained by Jeuffroy et al. (2013) who reported that legume crops have 5 to 7 times lower CO₂ emission per unit area compared to wheat, rapeseed and dry peas.

Further on, it was shown that legume crops can increase the bacterial activity in the rhizosphere by releasing hydrogen gas through biological N fixation procedure, which may indirectly influence and increase the SOC concentration (Golding and Dong, 2010). In addition, higher organic carbon concentration (7.21 g kg⁻¹ DM) was reported under grain legumes cultivation system compared to oat (Hajduk et al., 2015).

A further and very important reason for the positive effect of clover or field bean can be explained by the increase in the N accumulation and N content in the soil. The higher N availability results in higher biomass production (also in subsequent crops) and subsequently in increasing the SOC stock of the soil. In this regard it is shown that the intercropping of legumes like soybean combined with maize led to 26.6 g C kg⁻¹ increase in SOC accumulation compared to maize alone (21.8 g C kg⁻¹), therefore, soybean residue management promotes the SOC accumulation by 38.5% (Bichel et al., 2016). The N provision effect of legume pre-crops in cropping systems is an inevitable fact (Peoples et al., 2009; Preissel et al., 2015). Further findings from Angus et al. (2015) have shown the higher agronomic yield of wheat after grain legumes compared to pure cereals, which is attributed to the accumulation of N under legume management system. In addition, it must be considered that the performance of legume crops for biological N fixation and the provision of nitrogen to the soil differs between the legume species. For example, Peoples et al. (2009) reported that fava beans accumulate more N in their above ground biomass (153 kg N ha⁻¹) than field pea (130 kg N ha⁻¹).

A further aspect is the C/N ratio. Thus, it has been explained that cereal roots and stubbles have slower decomposition rate, which is mainly linked to their higher C/N ratio (Lorenz and Lai, 2005). Therefore, the lower N concentration in wheat roots and stubbles can reduce the microbial activity and the decomposition rate of the organic residues in the soil, which subsequently makes the wheat or cereals more efficient for retaining the C in the soil (Potter et al., 1998). Alternatively, the more available mineralized N led to increase the soil microorganisms and their activity, which in turn improves the soil rooting depth and structure (Drinkwater et al., 1998; Ross et al., 2008).

Another reason for the positive effect of legumes in cereal dominating crop rotation as found in the current study is a plant-pathological aspect. Pathogens and non-mobile pests in the soil are particularly susceptible to the crop rotation (Peters et al., 2003). Thus, different land use management (including legumes and cereals) with different plant morphology and nutrition demands can be breaking the chain of infection pathogens in the cropping system. As the cereals plant are the host of saprophytic pathogens (Paulitz et al., 2010) introducing legumes to the cropping system can be a suitable biological control of plant diseases. Thus, legume crops by increasing the beneficial microbes in the soil play an important role for degradation of saprophytic pathogens and interrupting the chain cycle of the soil borne disease in the soil.

Further on, it is explained by Elsen et al. (2008) that also mycorrhizas may play a crucial role in regulating of the soil borne disease and it provides protection against pathogens. Thus, it was observed by Johnson et al. (2013) that plants surrounded by greater diversity of microbes in their root structure have greater fitness and resilience against pests and pathogens. In addition, different land use management provide higher pathogen suppressive microorganisms and increase soil microbial biomass activity through increase of soil carbon by root exudates and residues compared to monocultures (Peralta et al., 2018).

The positive influence of legume previous crops on subsequent cereal crops has been examined and confirmed already several times in the past. For example, Evans et al. (2003a) found that the yield of first crop wheat that followed pea or forage vetch crops significantly increased compared to non-legume (cereal) crops. They indicated that wheat yields after clover or green manures are higher compared with grain pea so that the yields of subsequent crop wheat were significantly higher after green manure legume than grain legumes using as a break crop. A similar effect was observed in another study from Sheaffer et al. (2001) who found that legumes increased cereal yields more when managed for green manure than for grain. Benefits from 'break crops or pre-crops are more eminent in cereal-base rotations, where disease are the limiting factor of the yield production in cereal cultivations. Thus, cereal-break crops management system is reported to yield on average 24% more than cereals grown continuously in Northern Europe (Kirkegaard et al., 2008). In a study from Wittwer et al. (2017) it was found that the mechanism of biological N fixation by legume cover crops is responsible to enhance maize yield. They investigated maize as cover crop combined with legume species (legume and mixture) and have found significantly increased maize yield over the four years production systems.

Also, in a study from Sieling and Christen (2015) it was found that the rotation of wheat with peas or winter oilseed rape resulted in higher biomass yield and nitrogen uptake by the crop. Whereas, the cultivation of wheat or barley in a rotation significantly decreased the yield performance and N uptake of the crop. The higher approach in wheat yield under organic and inorganic fertilization can be related to the increase of soil nutrient availability derived from the decomposition of organic matter. Therefore, the combination of mineral and organic fertilization resulted in increase of SOM (Shen et al., 2011). Grain legumes improve growing conditions and increase the yield of subsequent crops in the rotation, an effect that has been analysed also in reviews from Köpke and Nemecek (2010) and Peoples et al. (2009).

The advantages of a mulching process, which was also used in our own LTE "BNF" Giessen, have already been described in the literature. Thus, Evans et al. (2003b) explained the net effect of N fixation on soil N balance was larger from green manure than from grain or forage conservation legumes. Therefore, it could be further postulated that legumes grown for green manuring might stimulate yield increases for more than one legume crop in the rotation. Secondly, the quantitative distribution of mineral N down the soil profile is varying depending on the crop species. For example, in the own LTE "BNF" it is revealed that clover mulch adds more N to the soil (see chapters 5. 1. 3), which improves soil fertility but is also beneficial for plant growth and biomass production.

Further on, in the current study was found that among all preceding crops clover mulch dedicated the higher NUE values in winter wheat in 2015 and in 2019 grown in the first year after pre-crops (see chapter 5. 2. 4) that postulates more N provision to the soil. Therefore, its effect is not only over the first year but also with the lower impact in the third year of crop rotation. Therefore, the positive effect of mulching system on SOC (see chapter 5. 1. 3), SMB C (see chapter 5. 1. 6) and the improvement of soil properties by crimson clover should be taken into account.

A further effect which was observed in the current study in 2015 was a synergistic effect among NPK fertilization and pre-crops on biomass yield of wheat in the first year of crop rotation. The increasing trend of biomass yield by adding N to PK 50% was lower after clover mulch than after field bean indicating higher agronomic efficiency of the land use under crimson clover as less N input is needed to increase the biomass yield to the maximum level. This increasing effect can also be explained with the biologically fixed N by clover mulch, therefore, and additional N inputs to the cropping system improve the nutrient assimilation in the crops and subsequently led to increase the biomass production.

Harvest Index (HI)

As a further plant parameter in the LTE "biological nitrogen fixation", the Harvest Index (HI) was determined five times during 2015 to 2020 to clarify whether changes have occurred within the above-ground biomass fractions of the cereals. Thus, only in 2017 a significant effect of preceding crops was found on HI of summer barley within both crop rotations (9th and 10th crop rotation). Despite, mineral fertilization significantly increased the HI of winter wheat (2015 and 2019), summer barely (2017) and winter rye (2020). The additional N input led to slightly increase in HI of wheat 2015, from 0.57 (90 kg N ha⁻¹) to 0.59 (180 kg N ha⁻¹) while this difference was larger in field bean, oat and maize. The same patterns were observed within the 10th crop rotation. Hence, in the 2nd and 3rd year of crop rotation after cultivating previous crops the variability of HI declined in winter rye and summer barley. This result can be associated with the N provision by crimson clover in the cropping system as also reported by Ross et al. (2009). However, in winter rye (in 2016) no positive effects were found between different levels of nitrogen fertilization. This can be possibly due to the fact that excessive soil N content, which in turn delay or reduce the soluble carbohydrate fractions of wheat residues including tillers, stubbles and leaf. This tendency also promises the higher carbon accumulation and confining the vegetative carbon mobilization to grain resulting in decreasing the HI (van Herwaarden et al., 1998).

Further on, it was also observed that fallow land within both crop rotations investigated here has increased the HI with the same pattern as crimson clover in crop rotation system. This trend can be caused by the higher mineralization rate of N in fallow land compared to under covered lands as also reported by Campbell and Paul (2008).

5. 2. 4 Nitrogen use efficiency and NPK uptake by winter wheat, winter rye and summer barley

Nitrogen use efficiency (NUE)

Nitrogen (N) is a major driver in crop production. Nitrogen management in agriculture aims to achieve agronomic (farm income, high crop productivity) and environmental objectives (minimal N losses) simultaneously. Nitrogen use efficiency (NUE) and their components are indirect measurements of the sustainability of cereals production systems (Santillano-Cázares et al., 2018). Increasing the N uptake and accumulation in the agronomic yield efficiently improve the NUE, while reducing the decomposition of N fractions (Moitzi et al., 2020). Zhang et al. (2015) reported N output-input ratios of 0.52 kg harvest N kg⁻¹ input N in Europe.

N use efficiency is governed by three major factors, which include N uptake by the crop, N supply from soil and fertilizer, and N losses from soil-plant systems. The crop N requirement is the most critical factor influencing N use efficiency (Ladha et al., 2005). An efficient N use is therefore a key indicator in sustainable plant production (Moitzi et al., 2020). In convergence, potential of retrieving N by the harvested product to the rate of N application define as N fertilizer efficiency (Barraclough et al., 2010). Thus, both NUE and N supply efficiency are strongly associated with the N absorption potential of roots and its translocations and accumulations into the shoots (Ladha et al., 2005).

Olesen et al. (2007) have indicated that non-legume crops which are combined with legumes in one rotation will benefit from the nitrogen released by crop residues returned to the soil. Thus, for example in a green manure system with grass-clover the most available N inputs obtained from biological N fixation by incorporating the grass-clover pasture into the soil. Similar inputs were obtained from crop residues of grain legumes and from catch crops. Hence, preceding crop residues govern the soil properties. Thus, it is explained that soil acidity adversely affects some morphological, physiological and biochemical processes in plants and thus also modify N uptake and N use efficiency (Fageria and Baligar, 2005; Marschner, 2011).

In the own study it is revealed that across all preceding crops the land use which includes clover mulch dedicated the higher N use efficiency values in winter wheat 2015 and 2019 in the first year after cultivation pre-crops. The variability between different previous crops was lower in optimal dosage of mineral fertilization than in split dosage. The lower N use efficiency value of soil (NUE_{soil}) under clover mulch compared to other preceding crops indicate that lower N input is required to achieve the optimal agronomic yield. It was found that the land use under 100% cereal management (oat and maize combined with subsequent winter wheat, winter rye and summer barley) required double or more N input to reach the same biomass yield as after clover mulch. That means on fertile soils or after excellent pre crops the N input is lower to get the same yield level compared to unfavourable pre crops. Alternatively, after unfavourable pre

crops like maize more N is needed to reach the same yield level. This result is according to the investigation of Marini et al. (2020) who found that continuous cereal cultivation (the land use with the proportion of 100% cereal) led to decrease the agronomic yield.

Furthermore, in winter rye in 2016 the N use efficiency was improved by preceding crops compared to fallow land. Conversely, in winter rye 2020 no improvement was observed between pre-crops. So, in 2020 the N provision and the effects of crimson clover were the same as maize. As rye is in the second year after the previous crops, the differences in the soil (N_{min}, SMB C) caused by the previous crop may have weakened during this period. However, this would mean that the previous crops in this trial do not yet have a sustainable lasting effect on the N use efficiency of the subsequent crops.

In addition, this result can also be caused by the N provision from mineral fertilizer or the N demand of cereals (wheat) to use the available mineralized nitrogen in the soil and the draining of N sources from previous crops. This effect was also explained by Gabriel and Quemada (2011), who noticed that legume and non-legume crops in a cover crop system do not influence the NUE compared to the bare land, although the main crop captures more N after legumes using as pre crops than fallow lands. This indicated that other sources of N like fertilizers act more efficient for the capture of N by the plant. Hence, the potential of cover crops on the NUE emerged when the fertilizer application is below the crop's N requirements. This is relevant because it allows to optimize the N fertilization of the cover crop.

Further on, the differences in NUE between winter wheat and winter rye can also related to the specific characteristics of both crop species. For example, rye has earlier plant development, higher vegetative shoot formation and a denser root system compared to wheat. Thus, also Hirel et al. (2007) explained for the number of crops, nitrogen absorption efficiency and N utilization efficiency is controlled by the gene variability. Therefore, from the relationship and performance of gene variability to the N levels, it can presume, that the more efficient variants under optimal or higher levels of N fertilization do not inevitably present the same performance under deficit N condition. In fact, this characteristic addressed the breeding traits of high-performance variant under the optimal or high nutrient availability that confine emerging of variants with low nutrient tolerant or low mineral or organic N fertilization conditions (Andrews et al., 2009).

In the own study the N uptake by plant shoot was significantly increased within both crop rotations (9th and 10th crop rotation) by clover mulch or mineral N input to the cropping system. So that by duplicating the N input from 90 to 180 kg N ha⁻¹ 41% increase of the N uptake was observed in winter wheat (2015) under maximal level of NPK fertilization. Winter rye and summer barley also showed the same response to the N input and the N provision by clover mulch in the crop rotation. Crimson clover increased the N uptake of winter wheat by 160.3 kg ha⁻¹ (66%) in the second year and of winter rye by 101.8 kg ha⁻¹ (11%) in the third year and of spring barley by 74.8 kg ha⁻¹ (13%) in the fourth year of the crop rotation compared to maize as an unfavourable preceding crop. A similar result was observed by Tonitto et al. (2006) and Quemada et al. (2013). They noticed that cover crops recover the soil properties and improve N balance in the system. In this context it is indicated that catch crops can be utilized as biological tools to improve the nitrogen economy of crop rotations (Thorup-Kristensen et al.,

2003). The accumulated nitrogen in catch crops can be suitably managed by the incorporation of residues into the soil. This also regulate the decomposition rate of the residue for the subsequent crops. Thus, the incorporation management including the timing and the amount of organic residues are very crucial to provide the nutrient for the requirements of subsequent crops.

P uptake

Phosphorous is an essential plant nutrient which is absorbed by the plant roots in form of HPO_4^{2-} and H_2PO_4^- (Schachtman et al., 1998). In many practices especially arable lands the application and management of phosphorous is very critical to ensure the production and increasing the potential yield of the crop. However, the uptakes of P by plants are not efficient enough during the vegetation period, which results in losses of more than 80% P by leaching or drainage down to the soil profile and bounded with the soil colloids and becomes unavailable for crops (Holford, 1997). In arable management systems soil P levels are regularly higher than in grass lands or wild ecosystems because of the higher P inputs, in such condition when the P level supply the requirements of the crop the reactions to P fertilization can be restricted (Scanlan et al., 2015).

It is acknowledged that P uptake by crops is increased by organic fertilizers along with mineral N inputs. This was also confirmed by Bauke et al. (2018) who have stated based on a long-term trial, that crop growth and microbial activity are boosted by mineral and organic NPK inputs. Bauke et al. (2018) showed that the optimal use of subsoil P gained only by the optimal application of NPK fertilization. Thereby, increased P export occurred by increasing crop yields by N fertilization. Alternatively, the organic acids will increase through root exudation as a result of increasing N fertilization to reach the maximum yield (Olander and Vitousek, 2000). This effect can be higher or lower under different land use management and genotypes and mycorrhizal activities (Jones et al., 2004; Lambers et al., 2002). Such root exudates improve P solubilisation for crop uptake. Deng et al. (2017) showed that the addition of organic fertilizer to the cropping system stimulate microbial activity and microbial P turnover.

Furthermore, it has been noted that P mineralization in the soil could be increased when other nutrients such as N and C are applied in sufficient amounts (Wang et al., 2021). Thus, the selection of cover crop species determined by their biomass production and potential in recovering nutrients for the subsequent crops. Using organic residues in management system not only provide nutrients as a requirement of crop growth, but also improves the soil chemical and physical properties (Manlay et al., 2007). Therefore, the nutrient richness of the organic residues like carbon, nitrogen and phosphorous also supports the soil microbial communities and increase their activity (Güsewell and Gessner, 2009). Generally, less mobility of P occurs under legume crops than other species due to their root exudates. The concentration of phosphatase, organic acids and ions observed to be higher in the soil managed by legumes (Li et al., 2007).

The own study has shown that the land use under which clover mulch could was included has increased the P uptake by subsequent crops in the first (wheat 2015), second (rye 2016) and third year (barley 2017) after cultivation of preceding crops. The identical increase (ca. 26

kg ha⁻¹) of P uptake by plants in the first and second year of crop rotation indicates the sufficient solubilisation of P input by mycorrhizal activity derived from crimson clover (Hallama et al., 2019) and the gradual decomposition of organic residues in cropping system provide and ensure the lasting effect of clover mulch on soil fertility within the second year should be taken into account. It is also showed that crimson clover and legumes have biochemical rhizosphere activity in which they release citrate that contributes effectively in P solubilisation and mobilization (Gerke and Meyer 1995; Li et al., 2007). This trend was reduced in the last year of cropping system by 16 kg ha⁻¹ approximately. Furthermore, N inputs at both levels improved the P uptake by plants through the crop rotation. Thus, it can be perceived that N input availability in the beginning of growing stage can positively affect the root development and distribution to the soil profile, which can in turn facilitate inter- and intra-specific competition in populations in favour of cash crop.

Legumes as a good P scavenger not only absorb the surplus P in the soil, but also provide adequate source of N to the cropping system. In this regards Ohm et al. (2017) reported that the labile P fractions in the arable soils after growing clover grass were relatively high. It can be stated that legume crops have a relatively high P requirement for their N₂ fixation. Also, less mobility of P in the soil have been reported in other studies (Talgre et al., 2014; Simpson et al., 2011). Therefore, the high labile P contents after red clover cropping systems, might be caused by P mineralization from crop residues or the mobilization of phosphorous (Talgre et al., 2014). The fact that the species with higher potential of P mobility in a rotation increase the P uptake and the growth of subsequent crops is well acknowledged (Simpson et al., 2011).

Tu et al. (2006) showed that N fertilization stimulate the growth of roots, which can explain the higher P uptake of the target crop by root extension and development in the beginning of the growing season. Furthermore, the subsequent decomposition of the roots may increase the organic P contents in the subsoil (Campbell et al., 1993). This result can explain how land use management or favourable cropping system can provide nutrient in rotation system.

Phosphatase enzymes contribute effectively in the regulation of microbial cycling of organic P in the soil (Richardson et al., 2011). Thus, increasing the amount of phosphatase may result in acceleration of releasing organic P from crop residues by microorganisms in the soil (Arruda et al., 2018). The performance of this fraction and the production of phosphatase in plant requires carbon and source of energy (Olander and Vitousek, 2000). It means the application of organic residues into the soil increase the microbial activity by provides more carbon and nitrogen thus more soluble organic P released into the soil (Wang et al., 2007).

In other study it is also noticed that there is a directional correlation between increasing the rate of N inputs and releasing organic P content of the soil due to the higher phosphatase activity (Olander and Vitousek, 2000). Alternatively, by increasing plant growth, the soil phosphorous accumulation decreases as a result of crop P requirements (Chen et al., 2018). Accordingly, in a long-term N management the higher P accumulation was found in subsoil (30–100 cm), where the low rate of N was applied (Bauke et al., 2018).

K uptake

Potassium (K^+) is a fundamental macronutrient for ensuring optimal plant growth (White and Karley, 2010) which fulfils critical physiological functions in the plant as it is related to enzyme activation, osmotic adjustment, cell expansion or stomata regulation in crops (Hawkesford et al., 2012). A large quantity of potassium is available in the soil in form of potassium silicates within secondary clay minerals (Huang et al., 2009) which therefore is difficult to absorb by the plant.

The absorption and utilization of potassium by crops is related to fertilizer types and the demands of the crops. More than 90% of the potassium is accumulated in vegetative organs (e. g. plant residues, straw) which can be released rapidly during the first 30 days of decomposition (Sui et al., 2017). Therefore, beside mineral K fertilization crop residue incorporation can be an effective method to improve soil K content (Singh et al., 2004).

It is well documented that K^+ is absorbed by High Affinity K^+ (HAK) transporters which will be activated when the K^+ concentration in the root surroundings is low (Gierth et al., 2005). After K^+ uptake it's accumulated in the vegetative tissue where the potassium content within the plant is much higher than in seeds. Hence, straw retention in the field can contribute a considerable amount of plant K to the soil.

Net positive or negative soil K balance have been observed when crop residues are either returned to the soil or removed (Whitbread et al., 2003). Whitbread et al. (2000) reported a K balance of $+8 \text{ kg ha}^{-1}$ when wheat straw was retained, and -102 kg ha^{-1} when straw was removed. Crop K uptake has been shown to increase when residues are retained (Mubarak et al., 2003). Thus, several studies have reported that the negative soil K balance under wheat-maize, soybean-wheat and other rotation systems can be significantly reduce by the application of crop residues (Bhattacharyya et al., 2006; Tan et al., 2007; Zhao et al., 2014).

In the own study it was found that the K uptake by plants was higher in treatments with clover mulch in the first (wheat 2015), second (rye 2016) and third year (barley 2017) of the crop rotation compared to fallow land, field bean, oat and maize. This effect can be caused by the higher crop biomass production under clover mulch and the higher K assimilation during the growing season compared to the other preceding crops.

This could be affected more by the crop species than by management practises. By monitoring the K uptake by the plants over the 9th crop rotation, it was found that the K uptake in winter rye is two times higher than in winter wheat (2015) and three times higher than spring barley (2017). This can be explained firstly due to the different plant species which can differ in their K uptake efficiency (White et al., 2021). A further reason can be explained by the extensive root system in rye which can be more efficient in taking up nutrients than wheat, barley and oat. Rye roots are able to recover K and other nutrients from deeper layers of the soil profile and translocate them to the upper horizons, where they become accessible to the subsequent crops (Valenzuela and Smith, 2002). Also, soil fertility status also affects K uptake by crops because the level of nitrogen in the soil enhances the K uptake in crops (Dotaniya et al., 2016). Further on, K availability in soil can be enhanced by the addition of the crop rotations with vigorous, deep-root system crops (Williams and Weil, 2004).

Lupwayi et al. (2005) explained that more than 90% of accumulated K in crop residue was released to the soil within one year after the addition, because contrary to N and P, K is not a structural component of plant tissue. In addition, the higher N provision by clover mulch compared to other preceding crops promote the growth and root extension of subsequent crops resulting in increase of nutrient uptake potential in the rotation system. Based on own findings, this assumption is also plausible for mineral N inputs to the cropping system, as the additional dosage of mineral fertilization (NPK 100%) has increased the K uptake of wheat up to 101% (50.2 kg ha⁻¹), rye up to 238% (125 kg ha⁻¹) and barley up to 48% (24.5 kg ha⁻¹) compared to control.

Zhao et al. (2014) reported a surplus of K only during NPK plus straw treatment. They suggested that crop straw or K fertilizer alone could not meet the K requirement for high crop yield performance and soil K balance. Thus, K fertilizer combined with the incorporation of crop residue is needed to match higher K removal rates and maintain long-term sustainability of the system, especially in low K fertility soil. In addition, crop residue and fertilizer K had different effects on soil net K. The K fertilizer significantly increase water-soluble K, but crop residues improve non-exchangeable K. This is due to the fact that basic part of K fertilizer is inorganic salt, which dissolves easily and taken up rapidly by the plant. In contrary, the organic K of crop residue assimilated and accumulated in cells and organs, and thus is only slowly released into soil (Wu et al., 2011b).

5. 2. 5 Thousand grain weight (TGW) and grain quality (protein and starch content)

Thousand grain weight

The thousand grain weight (TGW) is an important yield component of all grain crops, including cereals. It characterises the size of the caryopses of cereals, which are formed during the grain filling phase (from flowering to full maturity). The TGW values can vary greatly in wheat, rye and barley, as they depend on the air temperature and water supply during the grain formation phase. As a result, clear annual and site differences in TGW can be observed (Baillot et al., 2018; Li et al., 2016).

In addition, the TGW reacts to the different plant or stalk density of the plant stands in cereals. This means that lower TGW values are often formed at high culm (spike) density and vice versa. Other factors influencing the TGW are genetics (of the variety) (Matjar et al., 2018) and N nutrition (amount and timing of N fertilization) (Abedi et al., 2011; Tian et al., 2011).

Furthermore, it is known that a large caryopsis of wheat or other cereals represented by higher TGW contains more starch and has therefore often higher resources for the germination process. That can be explained by the fact that starch is enzymatically degraded into di- and mono-saccharides, which in turn serve as a source of nutrients for the embryo. Thus, stronger seedlings can form, which enable better plant development (developed primary root system and stronger above ground canopy) of the juvenile plants (Protic et al., 2013).

In the own study, no significant differences were observed among different land use managements in wheat 2015 and rye 2016. However, TGW values were slightly higher after

legume crops than after cereals or fallow land. In summer barley grown in 2017, clover mulch, led to increased TGW compared to maize and fallow. The same trend was observed by field bean and oat. Compared to the 9th crop rotation (2015-2017), more fluctuation was observed in wheat 2019 within the 10th crop rotation. Fallow land, field bean, maize and clover steady increased the TGW of wheat compared to oat as preceding crop. The large variation of TGW which was observed over the years, is possibly attributed to the influence of meteorological conditions, the occurrence of diseases and to physiological interactions within the plant stand.

The influence of plant residuals and organic fertilisation on TGW of cereals has also been studied by other researchers. Thus, Ehsan et al. (2014) studied the green manure managements on wheat crop and reported that incorporation of green manure led to highest TGW of wheat grown after rice. Further on, Agegnehu et al. (2014) observed that the utilization of organic and inorganic nutrient or the mixture of them led to significant effect on wheat grain yield, total biomass and HI, but not on its TGW. Also, Valkama et al. (2013) have reported in a meta-analysis that the response of TGW varied across the study and it decreased with increasing SOM content. They showed, N fertilizer may increase TGW by up to 10% in mineral soils with very low SOM content. It is further assumed that the incorporation of green manure in the soil in management system have a positive impact on wheat crop due to the N provision. The release of nutrients from green manures is rather slow, so it acts like a spontaneous supply of nutrients throughout the crop grows period compared to the utilization of mineral N (Hoque et al., 2016). Also, Holik et al. (2018) observed that application of NPK fertilization plus FYM led to higher TGW of winter wheat compared to control.

In the own investigation was observed that within the 9th crop rotation every mineral N input to the cropping system led to increase the TGW of subsequent cereal crops over the first (wheat 2015), second (rye 2016) and third (barley 2017) year of the crop rotation. This is a well-known effect which can be explained by better nutrition and assimilate supply of the ears and its seed grains. This relationship was also noted in several studies before (e. g. Protic et al., 2007; Litke et al., 2018, Ullah et al., 2018).

Conversely, in the current LTE “BNF” in 2019 the additional dosage of only 50% N has also increased TGW but higher N amount (100% N) has reduced the TGW of wheat. Furthermore, in rye 2020 no significant response was found by the utilization of mineral N fertilization. It is presumed that the decrease of TGW in wheat 2019 is attributed to intraspecific competition within the wheat plant stand. In that year (2019), by increasing N fertilization from 50% N to 100% N a very clear increase in ear density from 500 to 628 ears/m² was observed, respectively. This increase in ear density caused a decrease in water, nutrient and assimilate supply to the individual plant/ear or seed kernels within an ear due to the competition, which in this case led to a reduced TGW. This effect underlines the fact that the TGW of cereal crops like wheat is interrelated with the density of the plant stand and therefore does not always respond directly to N availability or N fertilization.

The negative response of TGW of wheat to N fertilization can also be related to the possible increase of grain number per unit area. By increasing the grain number per unit area, N inputs may concomitantly increase the number of grains in the distal positions of the spike or in spikes of secondary tillers (Acreche and Slafer, 2006; Gonzalez et al., 2005). Therefore,

these grains have a lower weight than proximal grains in the main shoot spikes. Thus, increasing grain number per unit area may reduce mean grain weight.

In general, it can be presumed that TGW is not a good indicator of cereal crop response to soil property change because grain size is the result of several interacting factors (genetics, weather conditions, N fertilisation and intraspecific competition within the plant stand).

Protein content in the seeds

Grain protein concentration is a basic indicator of grain quality in cereals particularly in wheat where it is widely used as the main parameter in evaluating baking quality of wheat products. However, the backing quality is very complex and determined not only by grain protein concentration but also by its composition as particularly by the protein fractions gliadin and glutenin (Xue et al., 2019).

Contrary to wheat, where protein quality and concentration plays a fundamental role in processing to baking products, for rye, starch with its properties (e. g. water absorption, viscosity, elasticity, extensibility) and, also pentosanes constitute the quality parameter (Laidig et al., 2017). Also, Hansen et al. (2004) stated that the role of rye protein molecules regarding bread baking is considered to be low. For this reason, the quality of rye is not determined by the protein, but more by the water absorption capacity of the pentosanes as well as the viscosity properties of the starch.

In barley, the third cereal species investigated in the current LTE “BNF”, grain protein content is closely associated with feed and malting quality. It’s acknowledged that a lower and moderate protein concentration is favourable for the malting properties of barley. Lower protein contents in the grain usually lead to higher starch contents, which in turn are important for a high malt quality, as low-molecular sugars are formed from the starch, which are needed for the subsequent yeast nutrition. Furthermore, a certain (moderate) protein concentration is also necessary because grain protein influences positively the yeast nutrition, haze formation in beer and enzyme activities (See et al., 2002; Clancy et al., 2003).

In general, grain protein concentration is determined mainly genetically. Further on, the improvement in grain protein concentration is also associated with climate condition and a narrow genetic variability was found within the gene pool wheat varieties (Iqbal et al., 2016). Further important factors influencing the protein content are the nitrogen uptake and supply of the plants (Zhang et al., 2016) as well as climatic conditions such as the available moisture during the grain filling phase of the plants (Vollmer and Mußhoff, 2018).

In the own study it was found that within the 9th crop rotation the land use with clover mulch significantly increased the grain protein concentration of wheat (2015) compared to all other preceding crops. This can be attributed to the N provision potential of legumes compared to the non-legume crops. Comparing both legumes (crimson clover and fava bean) the use of clover mulch as preceding crop was more efficient than field bean to increase the grain protein concentration of wheat as the subsequent crop. This is probably because clover mulch adds more C and N to the soil (see chapters 4. 1. 2 and 4. 1. 3), which improves soil fertility but is also beneficial for growth and protein accumulation in wheat.

Within the second year (2016) the grain protein concentration of rye was slightly higher after clover mulch but statistically identical with field bean and higher compared to the other preceding crops. Thus, the differences between the legumes in the first year of the crop rotation can be related to the amount of nitrogen in the crop residues, which seems to be larger after clover mulch and somewhat lower after field bean. Furthermore, it is presumed that the N release of clover mulch starts earlier in the course of the year and thus more N quantities are provided. It may also associate with the decomposition rate of the organic matter, which is faster with clover mulch than with field bean straw that contains more fibre.

Regarding the influence of legumes, it was shown in the literature that under dryland condition, winter wheat consistently had higher grain protein contents when grown after winter and spring grain pea grown than after fallow land or barley. However, the incorporation of green manure residues is more effective than legumes grown for hay or grain to increase the grain protein concentration of subsequent crops because green manure provides more N (Jones and Olson-Rutz 2012). In this respect, it can be generally stated that legume green manure fixes more N when their vegetation period is extended, especially in moist years. However, this must be regulated with the water requirement in the cropping system so that the subsequent crop yield is not decreased (Olson-Rutz et al., 2010).

Earlier termination of break crops retains more soil moisture and allows more time for the N to become available. Also, in the study from Evans et al. (2003a) was shown that the variability in grain protein concentration and grain yield of wheat after legumes was significantly correlated with variation in mineral N at wheat growing season. Further on, Kramberger et al. (2014) found after crimson clover as a preceding crop there is a richness of N for the subsequent maize cultivation.

In the LTE investigated in this study it was also observed that the lasting effect of preceding crops was reduced from the first (wheat) to the second (rye) and third (barley) year of the crop rotation. So that in the third year (spring barley) no significant differences were observed in grain protein concentration among preceding crops. Looking at the 10th crop rotation the same trend was observed as in the rotation before. The fixed and adequate mineralized N remained from clover mulch led to increase of protein concentration in wheat as first subsequent crop. In addition, the reduced trend in the second and third crop is possibly attributed to the N leaching and the N uptakes by the crops through the growing season.

In the current study it was also observed that every input of mineral N has increased the protein content of grains over the 9th and 10th crop rotation compared to control. This is a well-known effect which is attributed to the function of nitrogen for protein accumulation. Nitrogen is a basic part of amino acids which are the building blocks of protein necessary for plant growth and accumulating in the grain as a nutrient for the later germination of the embryo. Most of the N uptake by wheat plants used before heading or flowering stage for grain protein formation and translocated to the developing kernel during grain filling stage (Jones et al., 2011).

Alternatively, when the N inputs is not sufficient to the crop, decreasing protein concentration is inevitable to increase the grain yield due to the dilution effect (Jones and

Olson-Rutz 2012). Further on, it was found when the N inputs sufficiently support the requirement of crop to gain the maximum yield, the additional sources of N contribute to improve the protein concentration of the grain to the highest level (Fuertes-Mendizábal et al., 2010; Zhang et al., 2016).

In the literature it is confirmed that mineral N fertilization is more effective compared to organic fertilization, either in terms of biomass formation or grain protein accumulation (Černý et al., 2010; Tosti et al., 2016; Mayer et al., 2015). Further on, Černý et al. (2010) reported that winter wheat that received organic fertilization had up to 19% lower grain yields than that which was fertilized with mineral N. In another study conducted by Rossini et al. (2018) was shown that considering only N effects and the same N rate, mineral nitrogen fertilization achieved significantly higher grain yield and protein concentration of wheat compared to organic N, and these parameters increased with the increase of N rate.

In summary, it can be concluded that legume preceding crops increase the grain protein concentration of the direct subsequent cereal crop. This effect seems to be greater with year-round legume mulch than with grain legumes like field bean. This effect can also be observed in the second year and only to a lesser extent in the third year. Thus, it can be presumed that after a total of nine or ten crop rotation rotations, the clover-mulch had an increasingly sustainable effect on the protein content of the subsequent cereals.

Starch content of the seeds

Starch is the main storage carbohydrate of all cereals, which can vary in the range from about 55 to 75% in the seed grains, depending on the cereal species and the growing conditions of the plants (Wang et al., 2015, Hansen et al., 2004). Relatively low starch contents are formed in barley and rye, while wheat and grain maize can reach the highest starch contents of all cereals.

The development and distribution of starch granules which are accumulated in the endosperm have been shown to be highly genetically controlled (Peterson and Fulchner, 2001, Li et al., 2008). Climate condition, such as drought or air temperature during grain filling, also affect grain development and the starch granule size (Dupont and Altenbach, 2003). However, nitrogen is the most important plant nutrient that directly affects the grain starch content and its properties (Kindred et al., 2008; Wang et al., 2008). Further on, it was shown by Liang and Yu (2004) that the split application of K fertilizers can improve the supply of sucrose and therefore, increase the accumulation of starch in the seed grains of wheat.

In the own study in the LTE “BNF” it was found that the land use by crimson clover resulted in marginal lower grain starch concentration in winter wheat within the 9th and 10th crop rotation compared to the other preceding crops. This finding can be related to the higher N supply of crimson clover, which leads to an increase in protein concentration and conversely to a reduction in starch content in wheat grains.

Furthermore, in the second (2016 and 2020) and third (2017) year of both crop rotations in the BNF trial no significant variations have been observed among the land use management variants. This means that the mineral N amount in the soil and the soil effects caused by the

previous crop decreased significantly after two and three years respectively, so that they no longer had any effect on the starch content of the grains in the subsequent crops winter rye and summer barley.

In addition, it was found that every mineral N input to the cropping system by N fertilization led to decrease the grain starch concentration within both crop rotations. This expected result can be caused by mineral N supply in the grain filling phase of the cereals which firstly enrich the grain nutrition by increasing the grain protein concentration resulting in decreasing the starch content.

The negative correlation of N fertilization on starch has already been frequently studied and proven. Thus, Litke et al. (2018) have shown that starch content was significantly decreased by higher mineral nitrogen fertilization. Therefore, there is a negative relationship between protein and starch concentration in cereal grains. Further on, also Sung et al. (2008) have noticed that the accumulation of higher soluble sugars and lower starch contents correlate negatively with increasing nitrogen availability. That is confirmed by Ning et al. (2018) who have observed that the kernel starch concentration in maize at maturity was negatively associated with N availability.

Overall, it can be concluded that both the protein (increase) and starch (decrease) content of cereal seeds are directly influenced by the nitrogen supply of the soil by the leguminous preceding crops. This effect is more pronounced immediately in the following crop and is reduced or levelled out in the second and third following crop. These relationships should be taken into account in the production of quality cereals as for example in baking wheat, starch wheat and malting barley where the protein or starch content of the seeds is crucial for its quality and use.

6 Validating of the hypothesis and conclusion

Based on the results achieved from the LTE “Biological N Fixation” Giessen in 2015 to 2020, the hypotheses made can be validated and answered as follows:

Hypothesis 1: Cultivation systems with a proportion of 25% legumes within the crop rotation have led to an increase in the total carbon (Ct) and total organic carbon (TOC) and dissolved organic carbon (DOC) contents in the topsoil after at least nine crop rotations. This effect is highest in clover green-mulch and lowest in fallow. It should be clarified whether this change has also occurred in the sub soil till 90 cm.

The total carbon content (Ct) in the topsoil (0-30 cm) was influenced by the previous crop in only two of total of seven measurements. Clover mulch and field bean only tended to be superior on these two dates. For this reason, the hypothesis that the 25% legumes (represented by clover mulch or field bean) in the crop rotation contributes to C accumulation in the upper soil layer cannot be confirmed here. The same applies to deeper soil layers. On the other hand, mineral N fertilization caused a definite increase in the Ct content of the topsoil in five out of seven measurements. The hypothesis on the influence of N fertilization on Ct content can thus be confirmed.

Dissolved organic carbon (DOC) in the topsoil was highest after clover mulch and lowest after fallow land which reflects the release of water-soluble C compounds by plant residues and thus confirms the hypothesis. The combination of clover mulch as a preceding crop with N fertilization to subsequent cereal crops increases the content of dissolved organic C-compounds in the soil.

The total organic carbon (TOC) content of the topsoil was significantly lower after fallow land than after the previous crops. However, there were no confirmed differences between the respective preceding crops. The hypothesis, that fallow land has reduced the TOC was thus confirmed. However, the increase of TOC in legumes could not be confirmed.

Hypothesis 2: Cultivation systems with a proportion of 25% legumes within the crop rotation have led also to an increase of total nitrogen (Nt), total nitrogen bound (TNb) content, mineral nitrogen (NO_3^- N) and dissolved organic N compounds (DON) in the topsoil. This effect is highest in treatments with clover green mulch and lowest in soil of fallow land.

The previous crop, clover mulch, causes an increase in Nt content in the topsoil, which is also present one to two years after clover mulch, thus confirming the hypothesis. The clover mulch effect on Nt is stronger than the effect of field bean. Both legumes lead to more Nt in the topsoil than oats and maize. The fallow land causes a reduction of Nt in the topsoil, thus confirming the hypothesis. In contrary, the accumulation of Nt in the subsoil could not be clearly proven, as only one of four investigations found an increase in 30-60 cm due to clover mulch and a decrease due to fallow land.

The levels of total nitrogen bound (TNb) in the topsoil were clearly increased by clover mulch in both measurements that were carried out. That investigation, which was carried out

in all three soil layers, showed that this TNb increase was detected in the entire soil profile (0-90 cm). This confirms the hypothesis that legume mulch leads to an increase in TNb compounds in the soil. Clover mulch also leads to an increase in nitrate content in the soil profile (0-90 cm) one to two years later in subsequent cereals. This trend is usually also observed with field beans. When cereals are cultivated after fallow land, the lowest nitrate contents are always present in the soil, which confirms the initial hypothesis.

The dissolved organic N compounds (DON) did not follow a clear trend. In the fallow year, they were significantly higher under fallow land compared to the previous crops, while they were lower in spring under wheat after fallow. In contrast, the increased mineral N fertilization led to an increase in dissolved organic N compounds in the topsoil.

Hypothesis 3: Legume crops including clover mulch and field bean increase the cation exchange capacity (CEC) of the topsoil compared to cereals or fallow land after at least nine crop rotations.

The cation exchange capacity was not changed by the previous crops in the LTE "BNF". Therefore, the hypothesis that clover mulch and field beans led to the increase in CEC after at least nine crop rotations (incl. cultivation of legumes) must be rejected.

Hypothesis 4: Year-round greening with clover mulch and annual field bean cultivation which were repeated at least nine times in the cultivation increase the soil microbial biomass carbon (SMB C) in the topsoil.

In the current study the soil microbial biomass carbon (SMB C) in the topsoil was determined in total five times (five years). With one exception, the values after clover mulch were always in the upper range, but only once significantly higher than after other previous crops. The hypothesis that clover mulch increases SMB C can therefore not be clearly confirmed. Mineral N fertilization also led to a significant increase in SMB C in only one of five studies. Therefore, the assumed positive influence of N fertilization on SMB C cannot be confirmed either.

Hypothesis 5: The CO₂ efflux of the soil is influenced by seasonal effects (soil temperature) and by soil moisture. The use of fallow land and legumes in a rotational crop sequence increase the CO₂ efflux of the soil surface in the first and second subsequent year.

The near surface CO₂ efflux of the soil is strongly varying between the seasons. By increasing the soil/air temperature in the summer CO₂ emission increased and reached to its maximum level. In addition, the lower trend but significantly higher values were observed in spring and autumn compared to winter. Thus, the hypothesis that microclimate conditions in the soil directly influence the CO₂ efflux of the soil is plausible. Based on the result from a two-year campaign in LTE "BNF" it is well proved that the CO₂ efflux has a strong correlation with the soil/air temperature. Whereas a very weak correlation was found between soil CO₂ efflux and the soil moisture. Further on, no clear effect was found among different preceding crops. Thus, the hypothesis that legumes in rotational crop sequences increase the CO₂ efflux compared to fallow land must also be rejected based on the achieved result in LTE "BNF".

Hypothesis 6: Legumes (year-round green clover mulch and field beans) and mineral N fertilization led to an increase in the activity and abundance of the mesofauna of the topsoil layer in the ninth and tenth crop rotation, respectively.

Higher number of arthropods and higher feeding activity in the topsoil horizon was caused by the clover mulching system compared to fallow land or the land use under 100% cereal (oat – winter wheat – winter rye – summer barley). However, this trend was not sustainable among the mesofauna species, especially in the term of mites. Among the soil arthropods a positive tendency was found by collembolan species under the optimal dosage of mineral fertilization (NPK 100%). Thus, it is difficult to ascertain whether the soil arthropods react in the same way to the management circumstances because they are very dependable to their feeding habit and their compatibility to biotic and abiotic conditions in the cropping system. Therefore, the hypothesis that legumes and mineral N fertilization enhance the abundance of the soil fauna is partially plausible but either can't be generalized to all species and further seasonal investigations are needed to validate this hypothesis.

Hypothesis 7: The subsequent cereal crops react to the different previous crops and to the mineral N fertilization with an increase in vegetative growth indicated by higher leaf area index (LAI), vegetation indices (NDVI, NDRE), leaf chlorophyll (SPAD) and plant height.

The plant growth parameters like number of vegetative tillers, chlorophyll content of the leaves (SPAD), leaf area index (LAI), spikes density and plant height were significantly increased by mineral N fertilization as well as legumes used as preceding crops (mostly by clover mulch) compared to cereals or fallow land. In addition, it was observed that mineral N fertilization and green mulching (partly also fava bean) significantly improved the vegetation indices (NDVI, NDRE) of the subsequent crops in the first, second and third year after the pre crops. Thus, the hypothesis that the subsequent cereal crops (winter wheat, winter rye and summer barley) react to different previous crops and to mineral N fertilization with increased vegetative growth is plausible.

Hypothesis 8: Cultivation systems with a proportion of 25% legumes within the crop rotation have led to an increase of the biomass yields and harvest index (HI) in the first, second and third year of subsequent crops.

Biomass yields of subsequent crops were significantly increased by crimson clover especially in the 9th rotation in 2015 to 2017 whereas in the 10th rotation only in the first subsequent year (in 2019) a superiority of clover mulch was observed. Fallow land and oats, on the other hand, caused the worst growing conditions for the subsequent crops on average. As expected, the N fertilization caused a significant increase in biomass yields in all experimental years. Thus, the hypothesis that cultivation system with a proportion of 25% legumes within the crop rotation increase the biomass yield of subsequent wheat, rye and barley is confirmed in the current study of the LTE "BNF".

Further on, no significant effect was found among different preceding crops on harvest index (HI) of subsequent crops. Thus, the hypothesis that the contribution of legumes as previous crops increased the harvest index of subsequent crops must be rejected. In addition,

a positive and plausible effect of mineral N fertilization on HI of the cereal crops was found and confirms the original assumption.

Hypothesis 9: In addition to the direct effect of the mineral N fertilization, the legume previous crops also lead to a significant change in the grain quality indicated by an increase of thousand grain weight, protein content and volume weight and a decrease of grain starch content of the subsequent cereal crops.

Protein content of subsequent cereals is significantly increased by legumes as preceding crops as well as by mineral N fertilization. In contrary, the content of starch was reduced. Thus, the hypothesis that mineral N fertilization or legume previous crops lead to increase the protein content and reduces the starch content is confirmed in the LTE “BNF”. In addition, the thousand grain weight and volume weight of subsequent crops were increased by mineral N fertilization. Thus, the hypothesis that the mineral N fertilization causes the increase of the TGW, and volume weight of subsequent cereals is confirmed in the current LTE. However, there were no confirmed differences between the respective preceding crops. Also, sedimentation and gluten index values of winter wheat significantly increased by green mulching system as well as mineral N fertilization which confirms the original assumption.

Overall, it can be concluded that clover mulch (as a year-round green fallow land), after being repeated within nine crop rotations from the beginning of the current LTE investigated here, led to a significant change in the C stocks (represented by TOC and DOC but only in tendency by Ct) and N stocks (represented by Nt and TNb) in the topsoil (TNb also in subsoil). These changes were more pronounced under clover mulch than under field bean and can be assessed as sustainable. It can also be concluded that the mineral N fertilization carried out over more than three decades, presumably by promoting the root mass, contributes to increasing the C and N contents in the topsoil. Thus, mineral N fertilization also plays an important role in maintaining soil fertility.

The C/N changes in the soil have led to a higher biomass production of the subsequent cereal crops and led to a change of their NUE. In addition, it can be concluded that the plant growth parameters LAI, NDVI, NDRE and SPAD are well suited to demonstrate the reaction of the crop plants to changed soil properties even before harvest. These methods therefore represent reliable parameters to indicate the soil reaction of the plants at an early stage.

However, the year-round green fallow land does not provide crop yields and outcomes, when they are served as a conservation management to preserve the soil functions every fourth year of the crop rotation. In addition, clover mulch increases the likelihood that higher levels of nitrate will be formed as a result of the mineralization that prone to leaching during the establishment of subsequent crops.

Further on, it can be concluded, that the lasting changes in the soil caused by legumes can reduce the amount of mineral N applied and improve the conditions for the production of baking wheat, which requires higher protein and gluten contents.

Summary

The land use management has a major influence on soil properties and their functions. In this context, crop rotation, which is characterized by different plant diversity and mostly by high proportion of cereals also plays a major role. To compensate for the negative effects of crop rotations with a high proportion of cereals, leafy crops, legumes and catch crops, among others, are included in crop rotations that can have a long-term effect on soil properties and thus also on crop yields. Whether this is the case depends, among other factors, on the frequency and duration of cultivation of these crops and on the quantity and quality of the biomass added to the soil. The study carried out therefore aimed to clarify whether different land uses (fallow land, green mulch, field beans, oat, maize) that are part of cereal crop rotations have a lasting effect on soil fertility and on the biomass performance of the crop rotation.

To answer this question, the ongoing long-term experiment (LTE) "Biological N fixation" (established in 1982) at the Giessen site (Gleyic Fluvisol) was used and evaluated in its 9th and 10th rotation during 2015 to 2020. This LTE includes two test factors (1) crop rotation/preceding crops (fallow land, clover mulch, fava bean, oat, maize every fourth year followed by winter wheat, winter rye and summer barley) and (2) NPK fertilization (control, PK 50%, PK+N 50%, NPK 100%) with four field replications.

In 2016 to 2020 the soil was analysed for the following parameters: total carbon (Ct), CO₂ efflux (Fc), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), total nitrogen (Nt), total nitrogen bound (TNb), mineral nitrogen (Nmin), cation exchange capacity (CEC), soil microbial biomass C (SMB C), feeding activity of the mesofauna and its abundance. The measurements were carried out based on standardized methods usually according to VDLUFA and published laboratory instructions. In addition, growth parameters incl. vegetation indices and leaf chlorophyll (SPAD) as well as biomass yields, grain yield components, NPK uptake and grain quality of the cereal crops were analysed.

In the period from 2015 to 2020, after at least nine crop rotations, the following results were achieved: The Ct content in the topsoil tended to be higher after clover, followed by field beans, than for all other preceding crops in all years. However, only in two years in 2019 and 2020 out of seven measurements significant effects were found. According to achieved results, an increase in Ct due to clover mulch is very likely but could not be proven with certainty. The fallow land carried out every four years did not cause lower Ct values than the use of oats and maize. Mineral N fertilization, on the other hand, caused a clearer increase in Ct levels in the topsoil, which was proven in five out of seven measurements. It is thus concluded that the Ct increase also occurs via the larger root mass of the crops.

A Significant interaction effects between the main factors pre-crops and NPK fertilization was observed. Using the optimal dosage of mineral NPK fertilization (PK+100% N) led crimson clover to increase the DOC of the topsoil to the maximum level. In addition, also 100% NPK led to higher DOC level compared to the control. These results show that clover mulch as preceding crop with subsequent N-fertilized cereals led to increase in soluble carbon compounds, which contribute to the carbon dynamic in the topsoil.

TOC reacted positively to preceding crops as well as mineral N fertilization. It was observed that the higher dosage of N fertilization had a synergistic effect on mineralization of the organic matter residues on the soil surface. This effect was steady higher in treatments with clover mulch and oat compared to fallow land. Also, positive response was observed by mineral N input (PK+50% N and PK+100% N).

In the current study a strong seasonal effect of CO₂ efflux (Fc) of the soil was found. Fc values were positive correlated with the soil/air temperature and reached its maximum level in summer. In addition, a lower trend but significantly higher values were observed in spring and autumn compared to winter. However, no significant correlation was found between soil moisture and Fc. It was observed that preceding crop of clover-mulch tended to higher Fc values, which indicates a higher respiratory and microbiological activity of the soil. This trend was clearer in the first year compared to the second year after the preceding crops. Further on, it could be shown that mineral N fertilization increased the Fc of the soil surface compared to the control.

The microbial biomass (SMB C) of the topsoil was only significantly increased in the first year after the previous crops (2019) due to the effect of the previous crops themselves (sequence: clover-mulch/oats > field bean/maize > black fallow) as well as due to the mineral N fertilization. In other years, this effect only tended to be present or was absent.

The land use undertaken by crimson clover has led to higher population density of arthropods as well as higher feeding activity of the mesofauna in the topsoil compared to fallow land or the land use with a proportion of 100% cereals. This effect is attributed to the positive effects of the year-round clover-mulch variant (supply of above-ground and below-ground biomass with a narrower C/N ratio, lower evaporation of the soil, flattening of the temperature amplitudes at the soil surface).

The changes in soil fertility triggered by the preceding crops can already be detected during the vegetative growth of the following cereals. Suitable indicators of these plant responses were spike number, LAI, SPAD and NDVI. The positive effect of clover mulch could still be observed based on NDVI in the second and third year after the preceding crops. In addition, in four out of five experimental years, clover mulch caused the highest biomass yields in the subsequent crops. The lowest yields, on the other hand, were produced by fallow land and oats.

The previous crops / land uses also had an influence on the nitrogen use efficiency of the subsequent cereals. In the immediate subsequent crop, winter wheat, clover mulch causes a clear improvement in N utilization, indicated by higher NUE values. This effect was also observed in the 9th rotation in the second and third subsequent crop, winter rye and spring barley.

Overall, it is concluded that year-round clover mulch in a crop rotation led to sustainable changes in important soil fertility parameters after at least nine repetitions. The clover mulch effect was generally more pronounced than the effect of field beans, while fallow land and oats were the least favourable. These changes in the soil ultimately also correlate with N utilization, grain or biomass yields and the protein and starch content of the subsequent cereal crops.

Zusammenfassung

Die Art der Nutzung von Ackerböden und die darauf praktizierten Anbaumethoden haben einen großen Einfluss auf die Bodeneigenschaften und ihre Funktionen. Hierbei spielt auch die Fruchtfolge, die durch eine unterschiedliche Kulturpflanzen-Diversität und meist durch einen hohen Getreideanteil gekennzeichnet ist, eine große Rolle. Um die negativen Auswirkungen von Fruchtfolgen mit hohem Getreideanteil zu kompensieren, werden u.a. Blattfrüchte, Leguminosen und Zwischenfrüchte in die Fruchtfolgen integriert, die sich langfristig auf die Bodeneigenschaften und damit auch auf die Ernteerträge auswirken können. Ob dies der Fall ist, hängt unter anderem von der Häufigkeit und Dauer des Anbaus dieser Kulturpflanzen sowie von der Menge und Qualität der dem Boden zugeführten Biomasse ab.

Ziel der durchgeführten Studie war es daher zu klären, ob sehr unterschiedliche Formen der Bodennutzung (Schwarzbrache, ganzjähriger Klee-Mulch, Ackerbohnen, Hafer, Mais) im Rahmen einer 4-feldrigen Getreide-betonten Fruchtfolge nach acht bzw. neun Rotationen einen nachhaltigen Einfluss auf die Bodenfruchtbarkeit sowie auf die Erträge der Nachfrüchte innerhalb der Fruchtfolge haben.

Zur Beantwortung dieser Frage wurde der laufende Dauerfeldversuch (DFV) "Biologische N-Fixierung" am Standort Gießen (Bodentyp: Gley-Vega) genutzt und nach neun Rotationen in den Jahren 2015 bis 2020 ausgewertet. Der DFV beinhaltet zwei Prüffaktoren (1) Fruchtfolge/Vorfrucht (Vorfrüchte: Schwarzbrache, Klee-Mulch, Ackerbohne, Hafer, Mais, danach einheitlich Winterweizen, Winterroggen und Sommergerste) und (2) NPK-Düngung (Kontrolle, PK 50%, PK+N 50%, NPK 100%) mit vier Feldwiederholungen.

In den Jahren 2016 bis 2020 wurden folgende Bodenparameter untersucht: Gesamtkohlenstoff (Ct), CO₂ Efflux (Fc), gelöster organischer Kohlenstoff (DOC), gelöster organischer Stickstoff (DON), Gesamtstickstoff (Nt), gebundener Gesamtstickstoff (TNb), mineralischer Stickstoff (Nmin), Kationenaustauschkapazität (CEC), mikrobielle Bodenbiomasse C (SMB C) sowie die Fraß-Aktivität und Abundanz der Mesofauna. Die Messungen wurden nach Standard-Methoden nach VDLUFA sowie nach publizierten Labormethoden durchgeführt. Darüber hinaus wurden die Wachstumsparameter, die Biomasse-Erträge, die NPK-Aufnahme, die Ertragsstruktur und die Protein- und Stärke-Gehalte der Getreidearten bestimmt.

Es wurden folgende Ergebnisse erzielt: Der Ct-Gehalt im Oberboden war nach Klee-Mulch, gefolgt von Ackerbohnen, in allen Jahren tendenziell höher als bei allen anderen Vorfrüchten. Allerdings wurden nur in zwei Jahren von insgesamt sieben Messungen signifikante Effekte gefunden. Nach den erzielten Ergebnissen ist eine Erhöhung des Ct durch Klee-Mulch sehr wahrscheinlich, konnte aber nicht mit Sicherheit nachgewiesen werden. Die alle vier Jahre durchgeführte Schwarzbrache führte nicht zu niedrigeren Ct-Werten als nach Hafer und Mais. Die mineralische N-Düngung verursachte dagegen einen signifikanten Anstieg der Ct Werte im Oberboden. Daraus wird geschlossen, dass die Ct-Erhöhung im Boden auch über die größere Wurzelmasse der Kulturpflanzen erfolgt.

Es wurde ein signifikanter Interaktionseffekt zwischen den Hauptfaktoren Vorfrucht und NPK-Düngung festgestellt. Die optimale Dosierung der mineralische NPK-Düngung (PK+100% N) führte dazu, dass der Klee-Mulch den DOC-Gehalt des Oberbodens bis zum

Höchstwert erhöhte. Darüber hinaus führte 100 % NPK zu einem höheren DOC-Gehalt im Vergleich zur Kontrolle im Boden. Diese Ergebnisse zeigen, dass Klee-Mulch und die mineralische N-Düngung zu einer Erhöhung der löslichen C-Verbindungen im Boden führten, die zur Kohlenstoffdynamik im Oberboden beitrugen.

Der TOC reagierte positiv auf die Vorfrucht und die mineralische N-Düngung. Es wurde beobachtet, dass die höhere Dosierung der N-Düngung einen synergistischen Effekt auf die Mineralisierung der organischen Substanz im Oberboden hatte. Dieser Effekt war nach Klee-Mulch und Hafer im Vergleich zur Schwarzbrache stetig höher.

In den Untersuchungen wurde eine starke saisonale Variation des CO₂ Effluxes (Fc) des Bodens beobachtet. Die Fc-Werte waren positiv mit der Boden- bzw. Lufttemperatur korreliert und erreichten ihr Maximum im Sommer. Es wurde jedoch keine signifikante Korrelation zwischen Bodenfeuchte und Fc gefunden. Es wurde beobachtet, dass die Vorfrucht Klee-Mulch tendenziell zu höheren Fc-Werten führte, was auf eine höhere respiratorische und mikrobiologische Aktivität des Bodens hinweist. Dieser Trend war im ersten Jahr nach den Vorfrüchten deutlicher als im zweiten Jahr. Weiterhin konnte gezeigt werden, dass auch die mineralische N-Düngung den Fc-Wert des Bodens im Vergleich zur Kontrolle erhöhte.

Die mikrobielle Biomasse (SMB C) des Oberbodens war nur im ersten Jahr nach den Vorfrüchten (2019) sowohl durch die Wirkung der Vorfrüchte selbst (Reihenfolge: Klee-Mulch/Hafer > Ackerbohne/Mais > Schwarzbrache) als auch durch die mineralische N-Düngung signifikant erhöht. In anderen Jahren war dieser Effekt nur tendenziell bzw. nicht vorhanden.

Klee-Mulch hat zu einer höheren Populationsdichte von Arthropoden sowie zu einer höheren Fraß-Aktivität der Mesofauna im Oberboden im Vergleich zur Schwarzbrache und im Vergleich mit 100 % Getreide geführt. Dieser Effekt wird auf die positiven Wirkungen der ganzjährigen Klee-Mulch-Variante (Zufuhr an oberirdischer und unterirdischer Biomasse mit engerem C/N-Verhältnis, geringere Evaporation des Bodens, Abflachung der Temperaturamplituden an der Bodenoberfläche) zurückgeführt.

Die durch die Vorfrüchte ausgelösten Veränderungen der Bodenfruchtbarkeit lassen sich bereits während des vegetativen Wachstums der nachfolgenden Getreidearten erkennen. Geeignete Indikatoren für diese Pflanzenreaktionen waren Ährenzahl, LAI, SPAD und NDVI. Der positive Effekt von Klee-Mulch konnte anhand des NDVI auch noch im zweiten und dritten Jahr nach den Vorfrüchten beobachtet werden. Darüber hinaus verursachte Klee-Mulch in vier von fünf Versuchsjahren die höchsten Biomasseerträge in den Folgekulturen. Die geringsten Erträge bewirkten dagegen die Schwarzbrache und der Hafer.

Die Vorfrüchte bzw. Landnutzungen hatten auch einen gesicherten Einfluss auf die Stickstoffnutzungseffizienz der Getreide-Nachfrüchte. In der unmittelbaren Folgefrucht Winterweizen bewirkte Klee-Mulch eine deutliche Verbesserung der N-Verwertung. Dieser Effekt wurde in der neunten Fruchtfolge auch in der zweiten und dritten Folgefrucht (winterroggen und Sommergerste) beobachtet.

Insgesamt wird geschlussfolgert, dass ganzjähriger Klee-Mulch in einer Getreide-Fruchtfolge nach mindestens neunmaliger Wiederholung zu nachhaltigen Veränderungen

wichtiger Bodenfruchtbarkeitsparameter geführt hat. Die Wirkung von Klee-Mulch auf Bodeneigenschaften und Pflanzenertrag war generell ausgeprägter als die Wirkung von Ackerbohnen, während Schwarzbrache und Hafer am ungünstigsten wirkten. Diese Veränderungen im Boden korrelieren auch mit der N-Verwertung, den Korn- bzw. Biomasseerträgen sowie mit der Kornqualität (Protein- und Stärkegehalte) der jeweiligen Getreidearten in der Fruchtfolge.

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Appendix

Table A. 1: Effect of preceding crops (PC) and NPK fertilization (NPK) on CO₂ efflux (Fc) of the soil in vegetation periods in 2019, LTE “BNF” Giessen. Different letters indicate significant differences among the means; ns: not significant.

CO ₂ efflux (Fc) of the soil (µg C m ⁻² s ⁻¹)				
Date of measurement	Fallow	Clover	Oat	p-value
Autumn 2018 (in wheat)				
14 Nov. 2018	–	22.2 ^{ns}	23.9 ^{ns}	0.468
26 Nov. 2018	–	31.3 ^{ns}	28.6 ^{ns}	0.329
03 Dec. 2018	–	14.6 ^{ns}	19.9 ^{ns}	0.911
10 Dec. 2018	–	18.8 ^{ns}	16.8 ^{ns}	0.214
17 Dec. 2018	–	13.8 ^{ns}	13.6 ^{ns}	0.412
Spring 2019 (in wheat)				
16 Apr. 2019	42.0 ^b	52.9 ^a	50.0 ^{ab}	0.042
23 Apr. 2019	50.3 ^b	63.0 ^a	61.6 ^a	0.017
01 May 2019	44.6 ^{ns}	48.6 ^{ns}	48.6 ^{ns}	0.707
07 May 2019	34.7 ^{ns}	42.7 ^{ns}	45.8 ^{ns}	0.091
Summer 2019 (in wheat)				
15 June 2019	49.3 ^b	59.0 ^b	77.5 ^a	0.002
22 June 2019	62.4 ^b	52.9 ^c	72.4 ^a	< 0.01
29 June 2019	69.6 ^{ns}	56.1 ^{ns}	57.6 ^{ns}	0.338
06 July 2019	50.3 ^{ns}	38.2 ^{ns}	44.9 ^{ns}	0.185
13 July 2019	25.2 ^b	38.8 ^a	42.8 ^a	0.010
20 July 2019	39.3 ^b	51.6 ^a	59.5 ^a	0.001
27 July 2019	45.7 ^b	59.5 ^a	67.1 ^a	< 0.01

Table A. 2: Effect of preceding crops and NPK fertilization on NDRE 2016, LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Vegetation Index (NDRE)				
		2016				
		DC 32	DC 34	DC 60	DC 75	DC 82
Preceding crop (PC)	Fallow	0.38 c	0.17 c	0.16 c	0.16 c	0.14 ns
	Crimson clover	0.45 a	0.21 a	0.20 a	0.18 a	0.15 ns
	Field bean	0.42 b	0.19 b	0.18 b	0.18 a	0.15 ns
	Oat	0.43 b	0.20 ab	0.18 b	0.18 a	0.16 ns
	Maize	0.42 b	0.19 b	0.18 b	0.17 b	0.15 ns
NPK fertilization (NPK)	No fertilization	0.30 c	0.12 c	0.10 d	0.11 d	0.10 c
	PK 50%	0.32 c	0.12 c	0.11 c	0.12 c	0.10 c
	PK+50% N	0.47 b	0.24 b	0.22 b	0.21 b	0.17 b
	PK+100% N	0.59 a	0.30 a	0.29 a	0.26 a	0.22 a
p-value	PC	< 0.001	< 0.001	< 0.001	< 0.001	0.186
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.121	0.546	0.195	0.063	0.631
LSD 5%	PC	0.02	0.02	0.01	0.01	0.01
	NPK	0.02	0.01	0.01	0.01	0.01
	PCxNPK	0.03	0.03	0.02	0.02	0.03

Table A. 3: Effect of preceding crops and NPK fertilization on NDRE 2017, LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Vegetation Index (NDRE)					
		2017					
		DC 32	DC 37	DC 51	DC 56	DC 65	DC 75
Preceding Crop (PC)	Fallow	0.20 c	0.19 d	0.19 b	0.18 c	0.16 b	0.15 ns
	Crimson clover	0.25 a	0.24 a	0.23 a	0.23 a	0.19 a	0.17 ns
	Field bean	0.24 ab	0.23 ab	0.22 a	0.21 b	0.19 a	0.17 ns
	Oat	0.23 b	0.22 bc	0.20 b	0.21 b	0.17 b	0.16 ns
	Maize	0.23 b	0.21 c	0.20 b	0.20 b	0.17 b	0.16 ns
NPK fertilization (NPK)	No fertilization	0.21 c	0.18 c	0.17 c	0.16 c	0.14 c	0.13 c
	PK 50%	0.19 d	0.18 c	0.16 c	0.16 c	0.13 c	0.13 c
	PK+50% N	0.24 b	0.23 b	0.22 b	0.22 b	0.18 b	0.17 b
	PK+100% N	0.28 a	0.29 a	0.28 a	0.29 a	0.25 a	0.21 a
p-value	PC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.937	0.804	0.921	0.891	0.963	0.586
LSD 5%	PC	0.02	0.02	0.02	0.02	0.02	0.01
	NPK	0.01	0.01	0.02	0.02	0.02	0.02
	PCxNPK	0.03	0.03	0.04	0.04	0.04	0.03

Table A. 4: Effect of preceding crops and NPK fertilization on NDRE 2019, LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Vegetation Index (NDRE)						
		2019						
		DC 26	DC 31	DC 32	DC 50	DC 64	DC 72	DC 89
Preceding Crop (PC)	Fallow	0.22 b	0.27 b	0.29 b	0.31 b	0.29 b	0.23 b	0.09 a
	Crimson clover	0.26 a	0.35 a	0.36 a	0.39 a	0.36 a	0.28 a	0.10 a
	Field bean	0.21 b	0.27 b	0.29 b	0.32 b	0.30 b	0.23 b	0.09 a
	Oat	0.16 c	0.22 c	0.23 c	0.25 c	0.24 c	0.19 c	0.08 b
	Maize	0.22 b	0.27 b	0.28 b	0.30 b	0.29 b	0.22 b	0.09 ab
NPK fertilization (NPK)	No fertilization	0.19 b	0.23 c	0.23 c	0.23 c	0.23 c	0.16 c	0.06 c
	PK 50%	0.18 b	0.23 c	0.23 c	0.23 c	0.22 c	0.16 c	0.07 c
	PK+50% N	0.24 a	0.30 b	0.32 b	0.36 b	0.34 b	0.27 b	0.09 b
	PK+100% N	0.25 a	0.35 a	0.37 a	0.43 a	0.40 a	0.33 a	0.14 a
p-value	PC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.021
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PCxNPK	0.704	0.722	0.553	0.041	0.019	0.035	0.716
LSD 5%	PC	0.02	0.02	0.02	0.03	0.02	0.02	0.01
	NPK	0.02	0.02	0.02	0.02	0.02	0.02	0.01
	PCxNPK	0.04	0.04	0.04	0.05	0.04	0.03	0.02

Table A. 5: Effect of preceding crops and NPK fertilization on NDRE 2020, LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Vegetation Index (NDRE)			
		2020			
		DC 36	DC 51	DC 65	DC 73
Preceding crop (PC)	Fallow	0.098 c	0.118 b	0.127 cd	0.137 ns
	Crimson clover	0.126 a	0.136 a	0.146 a	0.128 ns
	Field bean	0.113 b	0.124 b	0.138 ab	0.133 ns
	Oat	0.114 b	0.123 b	0.134 bc	0.135 ns
	Maize	0.096 c	0.115 b	0.120 d	0.127 ns
NPK fertilization (NPK)	No fertilization	0.075 c	0.075 c	0.095 c	0.099 c
	PK 50%	0.070 c	0.077 c	0.083 d	0.096 c
	PK+50% N	0.127 b	0.149 b	0.151 b	0.149 b
	PK+100% N	0.166 a	0.191 a	0.203 a	0.184 a
p-value	PC	< 0.001	< 0.001	< 0.001	0.753
	NPK	< 0.001	< 0.001	< 0.001	< 0.001
	PC×NPK	0.308	0.097	0.647	0.563
LSD 5%	PC	0.01	0.01	0.01	0.02
	NPK	0.01	0.01	0.01	0.02
	PC×NPK	0.02	0.03	0.02	0.04

Table A. 6: Effect of preceding crops and NPK fertilization on grain quality parameters in winter wheat 2015 and 2019, LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Grain quality parameters (NIRS)					
		2015			2019		
		Spec.W. ¹ (kg hl ⁻¹)	Sed. ² (ml)	W. Glu. ³ (%)	Spec.W. ¹ (kg hl ⁻¹)	Sed. ² (ml)	W. Glu. ³ (%)
Preceding Crop (PC)	Fallow	74.12 bc	32.75 bc	19.9 b	75.71 ab	34.38 b	23.50 b
	Crimson clover	74.61 a	37.56 a	21.4 a	75.80 a	37.44 a	24.46 a
	Field bean	74.27 b	33.50 b	20.3 b	75.69 ab	33.56 b	23.15 b
	Oat	73.75 d	31.00 cd	19.4 b	75.10 c	33.69 b	23.17 b
	Maize	73.94 cd	30.69 d	19.4 b	75.23 bc	33.31 b	23.03 b
NPK fertilization (NPK)	No fertilization	73.69 c	28.85 c	18.6 c	74.13 c	25.40 c	20.34 c
	PK 50%	73.82 c	28.70 c	18.5 c	74.11 c	25.40 c	20.33 c
	PK+50% N	74.14 b	32.15 b	19.9 b	76.61 b	39.65 b	25.31 b
	PK+100% N	74.90 a	42.70 a	23.4 a	77.19 a	47.45 a	27.87 a
p-value	PC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PC×NPK	0.046	< 0.001	0.024	0.177	0.023	0.051
LSD 5%	PC	0.21	2.40	0.80	0.35	1.56	0.53
	NPK	0.18	2.14	0.71	0.32	1.39	0.47
	PC×NPK	0.41	4.79	1.59	0.71	3.12	1.06

¹ Specific weight (kg/hl); ² Sedimentation (ml); ³ Wet gluten (%)

Table A. 7: Effect of preceding crops and NPK fertilization on grain quality parameters in winter wheat 2015 and 2019, LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Grain quality parameters (NIRS)		
		2016		2020
		Specific weight (kg hl ⁻¹)	Falling number (s)	Specific weight (kg hl ⁻¹)
Preceding crop (PC)	Fallow	72.99 ns	307 ns	74.26 ns
	Crimson clover	73.38 ns	320 ns	74.39 ns
	Field bean	73.24 ns	323 ns	74.31 ns
	Oat	73.13 ns	304 ns	74.03 ns
	Maize	72.91 ns	310 ns	74.24 ns
NPK fertilization (NPK)	No fertilization	72.72 bc	312 ns	74.37 ns
	PK 50%	72.54 c	320 ns	74.01 ns
	PK+50% N	73.17 b	317 ns	74.07 ns
	PK+100% N	74.10 a	301 ns	74.55 ns
p-value	PC	0.157	0.194	0.672
	NPK	< 0.001	0.116	0.071
	PCxNPK	0.939	0.096	1.000
LSD 5%	PC	0.41	18	0.51
	NPK	0.37	16	0.45
	PCxNPK	0.82	37	1.01

Table A. 8: Biomass yield (grain and straw) of preceding crops - 2018, LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Biomass yield of preceding crops (dt DM ha ⁻¹) - 2018						
		CC ¹	FB ²		O ³		M ⁴	
		G + S	G	S	G	S	G	S
NPK fertilization (NPK)	No fertilization	59.9	8.0	11.5	55.8	61.5	46.6	70.1
	PK 50%	71.3	9.7	10.8	52.5	62.2	51.6	76.6
	PK+50% N	59.6	11.2	14.4	54.3	64.4	47.2	77.5
	PK+100% N	66.9	9.6	13.2	54.1	61.9	56.1	86.9
Mean		64.4	9.6	12.5	54.2	62.5	50.4	77.8
p-value	Fertilization	0.119	0.417		0.811		0.075	
LSD 5% dt ha ⁻¹	Fertilization	11.3	8.3		14.9		19.7	

¹ CC: Crimson clover (*Trifolium incarnatum* cv. Kardinal); ² FB: Field bean (*Vicia faba* cv. Hiverna); ³ O: Oat (*Avena sativa* cv. Fleuron); ⁴ M: Maize (*Zea mays* cv. Sunshinos). G: grain, S: straw.

Table A. 9: Total N Biomass yield of preceding crops (2018), LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Total N accumulated by pre-crops (kg DM ha ⁻¹)			
		2018			
		CC ¹	FB ²	O ³	M ⁴
NPK fertilization (NPK)	No fertilization	106.4	58.9	132.6	100.1 b
	PK 50%	128.9	66.8	126.4	107.0 b
	PK+50% N	118.8	80.5	131.9	99.6 b
	PK+100% N	111.5	73.0	139.1	131.7 a
Mean		116.4	69.8	132.5	109.6
p-value	Fertilization	0.477	0.425	0.162	0.039
LSD 5%	Fertilization	32.0	28.2	11.3	23.8

¹ CC: Crimson clover (*Trifolium incarnatum* cv. Kardinal); ² FB: Field bean (*Vicia faba* cv. Hiverna); ³ O: Oat (*Avena sativa* cv. Fleuron); ⁴ M: Maize (*Zea maize* cv. Sunshinos).

Table A. 10: Effect of preceding crops and NPK fertilization on total Ca uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Total Ca uptake (kg ha ⁻¹)		
		Winter wheat	Winter rye	Summer barley
		2015	2016	2017
Preceding crop (PC)	Fallow	2.44 b	2.35 b	1.64 c
	Crimson clover	3.37 a	2.82 a	2.01 a
	Field bean	2.68 b	2.46 b	1.88 abc
	Oat	1.64 d	2.50 b	1.73 bc
	Maize	2.07 c	2.51 b	1.97 ab
NPK fertilization (NPK)	No fertilization	1.84 c	1.61 c	1.73 b
	PK 50%	1.63 c	1.82 c	1.73 b
	PK+50% N	2.73 b	2.83 b	1.81 b
	PK+100% N	3.56 a	3.85 a	2.12 a
p-value	PC	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001
	PCxNPK	0.793	0.407	0.398
LSD 5%	PC	0.25	0.25	0.25
	NPK	0.23	0.22	0.21
	PCxNPK	0.51	0.49	0.36

Table A. 11: Effect of preceding crops and NPK fertilization on total Mg uptake by winter wheat, winter rye and summer barley (2015-2017), LTE “BNF” Giessen. Different letters indicate significant differences between the means.

Treatments		Total Mg uptake (kg ha ⁻¹)		
		Winter wheat	Winter rye	Summer barley
		2015	2016	2017
Preceding crop (PC)	Fallow	2.02 b	1.47 c	1.05 c
	Crimson clover	2.66 a	1.80 a	1.26 a
	Field bean	2.12 b	1.56 bc	1.19 ab
	Oat	1.39 d	1.56 bc	1.14 bc
	Maize	1.68 c	1.60 b	1.16 abc
NPK fertilization (NPK)	No fertilization	1.47 c	0.98 c	1.02 c
	PK 50%	1.40 c	1.09 c	0.98 c
	PK+50% N	2.25 b	1.85 b	1.23 b
	PK+100% N	2.79 a	2.49 a	1.41 a
p-value	PC	< 0.001	< 0.001	< 0.001
	NPK	< 0.001	< 0.001	< 0.001
	PC×NPK	0.062	0.077	0.068
LSD 5%	PC	0.152	0.10	0.12
	NPK	0.136	0.09	0.10
	PC×NPK	0.303	0.19	0.18

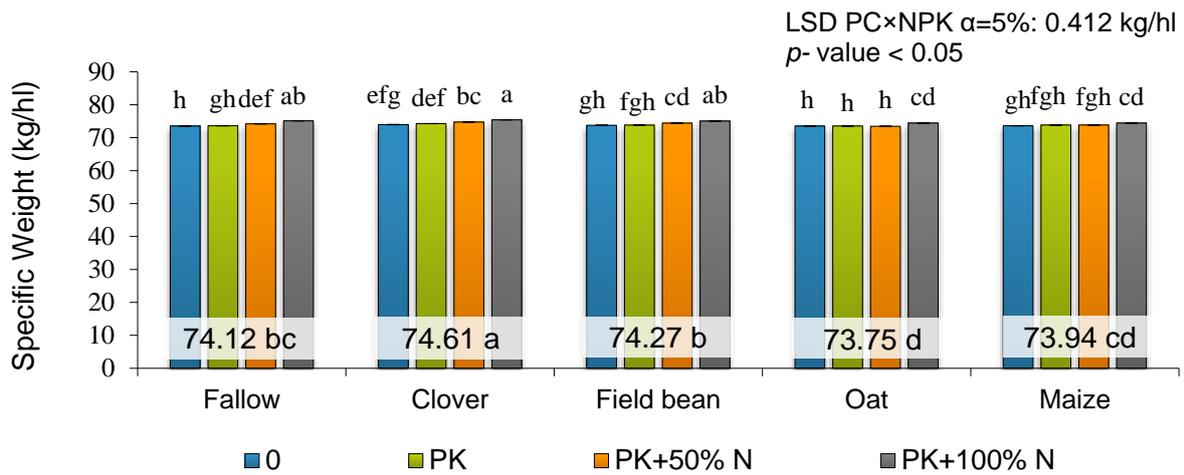


Figure A. 1: Effect of pre-crops (PC) and mineral fertilization (NPK) on specific weight of winter wheat's grain in 2015, LTE "BNF" Giessen. Different letters indicate significant differences between the means.

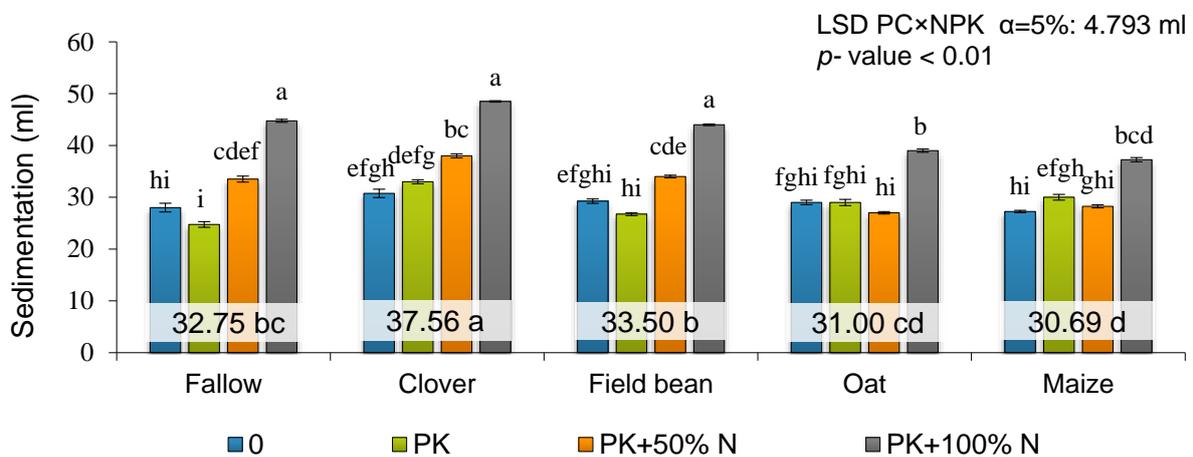


Figure A. 2: Effect of pre-crops (PC) and mineral fertilization (NPK) on sedimentation value of winter wheat's grain in 2015, LTE "BNF" Giessen. Different letters indicate significant differences between the means.

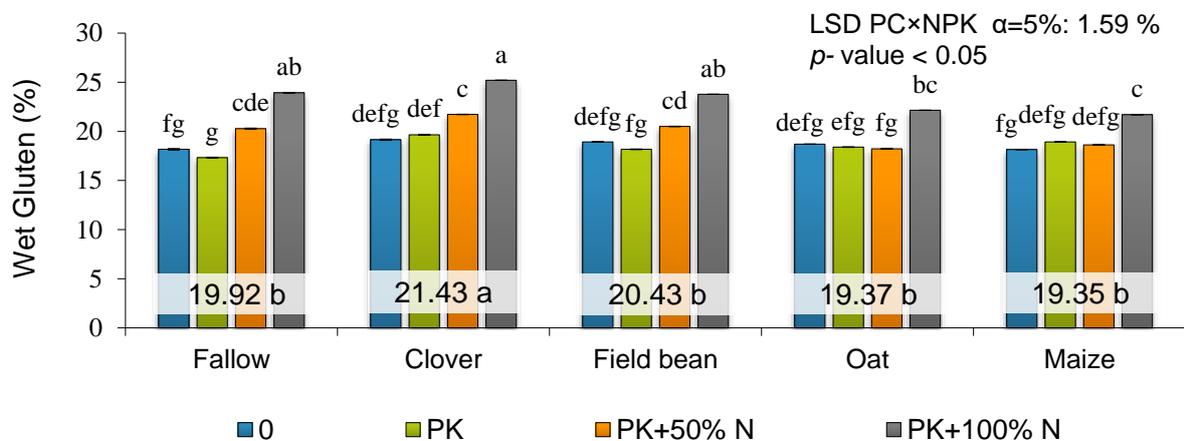


Figure A. 3: Effect of pre-crops (PC) and mineral fertilization (NPK) on wet gluten of winter wheat's grain in 2015, LTE "BNF" Giessen. Different letters indicate significant differences between the means.

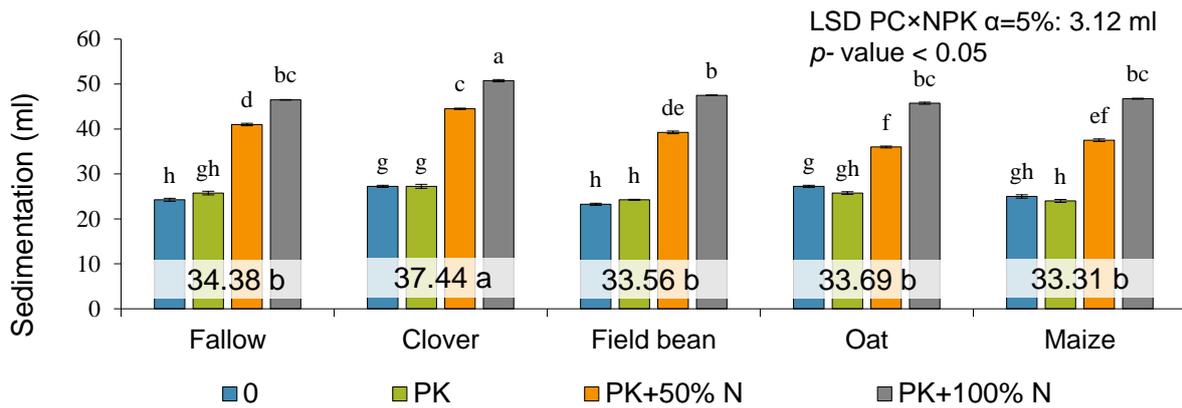


Figure A. 4: Effect of pre-crops (PC) and mineral fertilization (NPK) on sedimentation value of winter wheat's grain in 2019, LTE "BNF" Giessen. Different letters indicate significant differences between the means.

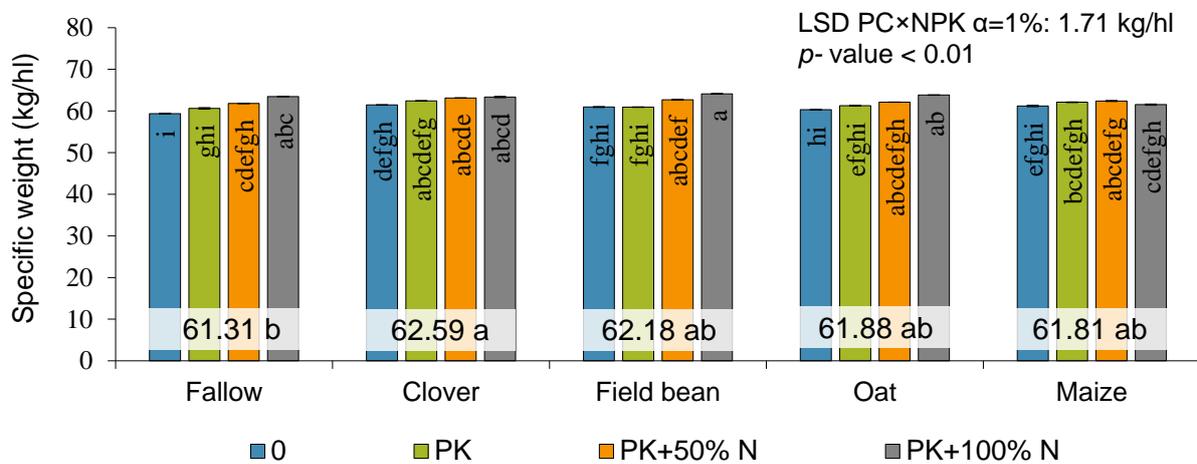


Figure A. 5: Effect of pre-crops (PC) and mineral fertilization (NPK) on specific weight of summer barley's grain in 2017, LTE "BNF" Giessen. Different letters indicate significant differences between the means.

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Declaration

"I declare: I have prepared the submitted dissertation independently and without unauthorized outside help and only with the help that I have indicated in the dissertation.

All text passages taken verbatim or in spirit from published writings and all information based on oral information are marked as such.

In the research conducted by me and mentioned in the dissertation, I have complied with the principles of good scientific practice as laid down in the "Statutes of the Justus Liebig University Giessen for the safeguarding of good scientific practice.

Place, Date

Yavar Vaziritabar