

# Transect sampling for soil organic carbon monitoring in temperate alley cropping systems - A review and standardized guideline

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## ARTICLE INFO

### Keywords:

Agroforestry  
Tree influence  
Soil sampling  
Sampling bias  
Multiple soil classes

## ABSTRACT

Agroforestry systems provide significant ecosystem services, including soil organic carbon sequestration. However, the structural complexity of these systems must be considered when taking samples. In particular, when sampling in the tree- and arable- or grassland strips of alley cropping systems (ACS), different levels of tree influence must be taken into account. The heterogeneity of soil sampling approaches in ACS has been creating challenges for comparability and the integration of findings in meta-analyses. Furthermore, some of the sampling approaches have led to biases of over- or underestimation of the tree influence for the whole system. We evaluated 48 studies of a published meta-analysis on soil organic carbon sequestration in temperate agroforestry systems, to identify, document and quantify potential biases associated with transect soil sampling in ACS. In all of the 23 transect designs evaluated in detail, at least one of the six identified biases was observed. Overall, 10 out of the 23 transect designs did not include soil sampling in the tree strip, which may lead to an underestimation of the tree influence. On the other hand, an overestimation of the tree influence may occur when the tree- and arable strips are not weighted for their respective area shares, which was considered only in three transect designs. To address the identified biases and enhance the accuracy and comparability of soil organic carbon analyses in ACS we propose a standardized guideline for transect sampling in temperate ACS.

## 1. Introduction

Agroforestry systems, i.e. trees or shrubs on arable or grassland with or without livestock, can play a crucial role for enhancing ecosystem services of agriculture (Torralba et al., 2016), for instance through increasing soil organic carbon (SOC) stocks and sequestration rates. This conclusion is supported by numerous studies (e.g., Cardinael et al., 2017; Pardon et al., 2017), including meta-analyses (Cardinael et al., 2018a; Chatterjee et al., 2018; De Stefano and Jacobson, 2018; Feliciano et al., 2018; Hübner et al., 2021; Ivezić et al., 2022; Kim et al., 2016; Mayer et al., 2022). Compared to other soil-based climate-friendly practices, carbon sequestration appears to be particularly high in agroforestry (Frelih-Larsen et al., 2022; Wiesmeier et al., 2020). Typically, studies compare SOC stocks or sequestration rates between agroforestry systems and non-agroforestry controls using paired sites (Chatterjee et al., 2018; De Stefano and Jacobson, 2018). These findings are used to inform researchers, practitioners, extensionists, policymakers, and the

public, and may be utilized by the Intergovernmental Panel on Climate Change (IPCC) for future carbon accounting purposes (Cardinael et al., 2018a). However, considering the soil sampling approach of the individual studies, the values obtained for agroforestry systems in these studies often do not appear representative of the entire system.

Agroforestry systems are characterized by a greater structural complexity compared to non-agroforestry agricultural systems (Nair, 1993). Soil processes in agroforestry systems are influenced by various factors that are known to decrease with increasing distance from the trees (or shrubs, always included hereinafter), such as litterfall (Oelbermann et al., 2006; Swieter et al., 2021; Swieter et al., 2019), shade (Blanchet et al., 2022), fine root distribution (Battie Laclau et al., 2020) and its associated rhizodeposition (Grayston et al., 1997). This results in pronounced gradients from the trees into the adjacent arable field, which was shown for SOC by Cardinael et al. (2018b) and for microclimate and water balance indicators by Jacobs et al. (2022). In addition to the trees themselves, the associated understory vegetation cover

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<https://doi.org/10.1016/j.geodrs.2024.e00757>

Received 24 April 2023; Received in revised form 29 December 2023; Accepted 9 January 2024

Available online 10 January 2024

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(Battie Laclau et al., 2020; D'Hervilly et al., 2020) and the absence of soil tillage around the trees influences the soil. Bai et al. (2018) showed that no-till management compared to tillage in agricultural systems leads to an increase in aggregate stability, soil organic matter content and earthworm populations. For instance, a higher earthworm density, likely reduced to the lack of tillage, was also shown in the tree strips of agroforestry systems by Cardinael et al. (2019) and Vaupel et al. (2023). Appropriately addressing this spatial complexity is critical for making accurate and meaningful general statements about agroforestry systems.

Several researchers have highlighted the challenges posed by the heterogeneity of soil sampling methodologies used in agroforestry research, which make data comparability and integration of findings across studies difficult (Cardinael et al., 2018a; Chatterjee et al., 2018; Feliciano et al., 2018; Golicz et al., 2022; Mayer et al., 2022; Nair, 2012). The main challenges reported by the authors are the lack of uniformity of sampling depths, sampling design description, baseline data or suitable non-agroforestry controls. Consequently, Chatterjee et al. (2018) and Golicz et al. (2022) highlight the need for standardization and the development of guidelines for SOC determination in agroforestry systems.

In 'modern' agroforestry systems in temperate regions, an alley cropping design is common. The tree strips are usually at a distance that allows for mechanized management of the arable or grassland strips with standard agricultural machinery (Nerlich et al., 2013). A widely used soil sampling approach in these alley cropping systems (ACS) involves the use of transects that cut through the tree- and arable strips (e. g., Cardinael et al., 2017; Golicz et al., 2023; Pardon et al., 2017; Upson and Burgess, 2013). The sampling method of transects is commonly used in soil field research (Pennock et al., 2006), ecological studies (Navarro and Díaz-Gamboa, 2014) or crop yield assessments in ACS (Swieter et al., 2019). However, there are challenges to using this approach in ACS concerning the comparability of studies. One example is the use of different tree strip distances, due to which Mayer et al. (2022) could not estimate the effect of tree distance on the SOC stock in their meta-analysis. This and the presence of several common biases underscores the importance of standardizing the transect sampling approach in ACS.

In this study, we identify potential biases that may lead to an over- or underestimation of the tree influence in ACS during transect soil sampling. The biases are described and discussed. Their occurrence is quantified based on a literature evaluation of soil sampling approaches used in studies analyzing SOC in temperate ACS. On the basis of these findings, a standardized guideline for transect soil sampling in ACS is proposed.

## 2. Materials and methods

### 2.1. Bias identification and guideline development

The study is based on expert meetings and an evaluation of the scientific literature on the topic of soil monitoring in ACS. Fifteen meetings and discussions with experts from the German Agroforestry Federation (Deutscher Fachverband für Agroforstwirtschaft, DeFAF) were conducted. All experts are scientists with a profound experience in soil sampling and agroforestry practice. The expert meetings led to the identification of potential biases in transect soil sampling and the development of the DeFAF guideline for soil monitoring in ACS (Minarsch et al., 2022) which forms the basis for this study. Rather than proposing a fixed protocol, the intention of the proposed guideline is to provide a toolbox with tested practices, which can be adapted for different research questions and ACS designs.

### 2.2. Study selection

To document different soil sampling approaches used in research on SOC sequestration in temperate ACS, all studies considered in a recent meta-analysis (Mayer et al., 2022) were used for a literature evaluation.

Of the 48 studies, only those that analyzed SOC in silvoarable (trees on arable land) or silvopastoral (trees with livestock) ACS were selected for data extraction. Twelve studies that focused on other systems including boundary plantings, hedges or shelterbelts, were excluded. Ten further studies were excluded because there was no clear indication of the tree distance for samples taken along a transect or at random locations. Another five studies that took samples only in the arable strip center or around a tree were excluded as well, as they did not use a transect sampling approach for the whole system (for a complete list see Supplementary Table 1). The remaining 21 studies were used for data extraction and identification of potential biases. Two studies used two different transect sampling designs for comparison, which were both considered individually in our assessment. One of these sampling designs examined a non-temperate ACS in Costa Rica. In total 23 transect designs were evaluated in detail (Table 1).

### 2.3. Terminology

For this study, the basic terms, processes, and transect types were defined as follows:

In ACS, trees (or shrubs, always included hereinafter) are planted in tree rows on tree strips (Fig. 1). The tree strips usually have an understory vegetation cover and lack soil tillage, due to the trees. They can be several meters wide and alternate with arable strips (or grassland strips, always included hereinafter). The width of the arable strips is, among others, defined by the working width of the available machinery used.

The tree influence, for example with regard to litterfall (Oelbermann et al., 2006), fine roots (Battie Laclau et al., 2020), rhizodeposition, shade (Blanchet et al., 2022) and windbreak (Böhm et al., 2014) declines with increasing distance from the tree (Fig. 2A). Therefore, we categorize the tree influence on its surrounding area into the four levels high, medium, low and zero. These should be considered within the tree strip, the adjacent arable strips and the different soil depths (Cardinael et al., 2017). The extent of the tree influence depends on tree-related factors, such as species (Vaupel et al., 2023), age (Cardinael et al., 2017; Golicz et al., 2023), height or crown shape and is further influenced by the topography, sun and wind exposition (Jacobs et al., 2022).

To cover the different levels of tree influence when assessing ACS, transects, which extend from the tree strip into the adjacent arable strip, are used for sampling. Depending on the type of transect, the sampling points can be located between two tree strips (T1), to one side of a tree strip to the center of the adjacent arable strip (T2), or to both sides of a tree strip towards the centers of both adjacent arable strips (T3, Fig. 2B). For transect type T2 two transect orientations are possible (Fig. 2C). For example, if the tree strip is oriented in a north-south direction, transects can expand towards the east and west sides of the tree strip. When trees in a tree strip are spaced several meters apart, different transect positions are possible, including next to a tree or in between two trees (Fig. 2D).

## 3. Biases in transect sampling

### 3.1. Transect orientation bias

The transect orientation defines if soil sampling is performed on one or both sides of a tree strip. This is of importance, considering that tree strips can have a sunny and a shady, a luv and a lee or an up- and a downhill side. This can result in differences regarding sun and wind exposition or soil runoff (Dupraz et al., 2018; Jacobs et al., 2022), which in turn can influence crop growth or litterfall and thus accumulation of organic matter (Swieter et al., 2021). If transects are oriented only to one side (or with unequal shares to both sides) of the tree strip, an under- or overrepresentation of the above-mentioned effects is possible. Based on the evaluated literature, seven out of the 23 transect designs used transect type T1 (between two tree strips) or T3 (to both sides of one tree strip), avoiding any orientation bias (Fig. 3A). The remaining 16 transect

**Table 1**  
Characteristics of transect designs used in alley cropping systems (ACS).

Transect design	Transect				Tree strip sampling					Arable strip sampling					CS	Depth [cm]
	Type	Nr.	Orientation	Position	Nr.	MTD [m]	Other	D/4	D/2	Nr.	MTSD [m]	Other	W/4	W/2		
Bambrick et al. (2010) a*	T1		N/A		0	N/A	N/A	N/A	N/A	3	0.75			yes <sup>#</sup>	C3	30
Bambrick et al. (2010)b	T2				0	N/A	N/A	N/A	N/A	3	1		yes	yes	C3	20
Beuschel et al. (2019)	T2	4			1					2	1			yes	C3	20
Beuschel et al. (2020)	T2	4			1					2	1			yes	C3	20
Cardinael et al. (2015)	T2	9	5 to 4	T	4	1		yes	yes	2	1	yes				200
Cardinael et al. (2017)	T2	3	2 to 1	T + B	4	1	yes	yes	yes	10	1	yes	yes	yes		60
Cardinael et al. (2019)	T2	3		T	2	1			yes	2	1			yes		30
D'Hervilly et al. (2020)	T2	5		B	2	1			yes	2	1			yes		20
Dube et al. (2012)	T3	3	N/A		0	N/A	N/A	N/A	N/A	8	2.5	yes	yes	yes		40
Gamble et al. (2020)	T2	4			1	0.5				3	1		yes	yes	C3	90
Gao et al. (2020)	T2	6	3 to 3	T	0	N/A	N/A	N/A	N/A	3					C2	200
Guo et al. (2020)	T2	3			1					4					C1	100
Lim et al. (2018)	T2	2			1					1						50
Oelbermann et al. (2006)a*	T3	6	N/A	T + B	0	N/A	N/A	N/A	N/A	3	0.75		yes	yes	C4	40
Oelbermann et al. (2006)b	T2	5			0	N/A	N/A	N/A	N/A	5	1	yes	yes	yes		40
Pardon et al. (2017)	T1	3	N/A	T + B	0	N/A	N/A	N/A	N/A	6	2	yes <sup>#</sup>		yes <sup>#</sup>	C3	23
Peichl et al. (2006)	T3	3	N/A		0	N/A	N/A	N/A	N/A	24	1	yes	yes	yes		20
Schmidt et al. (2021)	T2	4	2 to 2		1					3	1	yes				30
Seitz et al. (2017)	T2	5	5 to 0	B	1				yes	2			yes	yes	C3	60
Stöcker et al. (2020)	T2	4			1				yes	1	1.5					40
Upson and Burgess (2013)	T2				1	0.5				4	0.5	yes	yes	yes		150
Winans et al. (2014)	T1	6	N/A		0	N/A	N/A	N/A	N/A	3	0.25			yes	C3	30
Winans et al. (2016)	T1	6	N/A		0	N/A	N/A	N/A	N/A	3	0.7			yes	C3	30
<b>Number of observations</b>	<b>23</b>	<b>20</b>	<b>5</b>	<b>8</b>	<b>13</b>	<b>6</b>	<b>1</b>	<b>2</b>	<b>6</b>	<b>23</b>	<b>19</b>	<b>8</b>	<b>9</b>	<b>17</b>	<b>12</b>	<b>23</b>

Information provided refers to the transect, tree (TS) and arable strip (AS) sampling, composite sampling (CS) and maximum sampling depth. Transect types include T1 = between two TS, T2 = to one side of the TS and T3 = to both sides of the TS. Number (Nr.) indicates transects per system or samples per transect within TS and AS. Orientation indicates, e.g., that five transects are orientated west and four east (5 to 4). Position indicates sampling at: T = next to a tree, B = in between two trees or T + B = both positions. TS sampling points are defined by: MTD = minimum tree distance, D/4 = quarter tree distance, D/2 = half tree distance or Other = further locations. AS sampling points are defined by: MTSD = minimum TS distance, W/4 = quarter arable strip width and W/2 = half arable strip width. CS approaches include combining sub-samples: C1/2 = from the same transect, C3 = from the same sample point area and C4 = from two neighboring transects (Fig. 3C). Blank fields indicate that no information was provided and N/A = not applicable. Number of observations indicates the number of transect designs providing the information (N/A not included).

\* Bambrick et al. (2010): ACS named a) St. Paulin, St. Eduard, St. Remi and b) Guelph. Oelbermann et al. (2006) ACS in a) Costa Rica and b) Canada.

# The transect design used fixed sample distances for multiple ACS with varying arable strip widths.

designs used transect type T2 (only to one side of the tree strip). Out of these, only five reported the orientation of the transects used with the majority using unequal shares of both sides, resulting in an orientation bias.

### 3.2. Transect position bias

Transect positions should account for high, medium, low and zero levels of tree influence, in particular when trees in tree strips stand several meters apart from each other. An over- or underestimation of the tree influence can occur if the transects are only located next to a tree or in between two trees, respectively. Out of the 23 transect designs, only eight reported the transect position. Out of these, only three transect designs avoided a position bias by the use of positions both next to a tree and in between two trees for their transects (Cardinael et al., 2017; Oelbermann et al., 2006; Pardon et al., 2017). For all other transect designs with trees standing several meters apart in the tree strips a transect position bias might have occurred.

### 3.3. Arable strip sampling bias

To address different levels of tree influence in arable strips of ACS, soil samples should be taken at varying distances from the tree strip towards the center of the arable strip. An over- or underestimation of the tree influence is likely if samples are only taken close to the tree strip or only in the center of the arable strip. These arable strip sampling biases likely occurred in those transect designs that used a low number of samples (two and five transect designs sampled only in one and two locations, respectively) or in those transect designs that did not include sampling in the center of the arable strip (seven transect designs). In total, all transect designs included sampling in the arable strips with 1 to 24 samples per transect (median of 3). The average minimum distance between the first sampling spot and the edge of the tree strip was 1.1 m (median of 1.0 and standard deviation of 0.5). Additional sampling locations included the center of the arable strip (17 transect designs), half the distance to the center (nine transect designs) and other locations within the arable strip (eight transect designs, for observed sample locations see Fig. 3B).

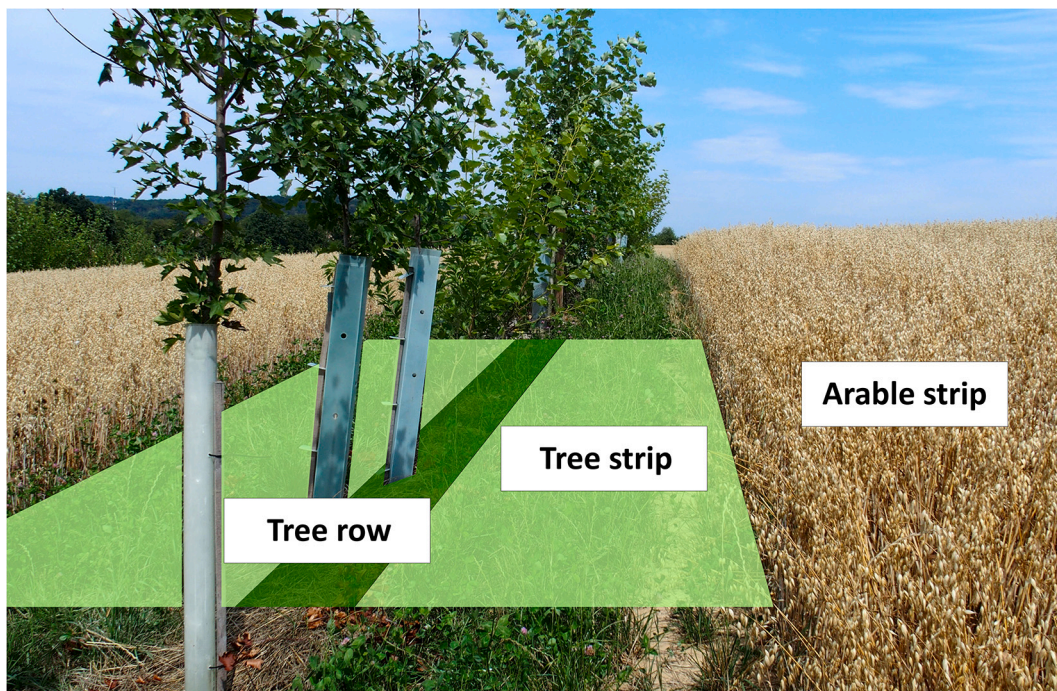


Fig. 1. Illustration of tree row, tree strip, and arable strip in an example silvoarable alley cropping system. Modified after Minarsch et al. (2022).

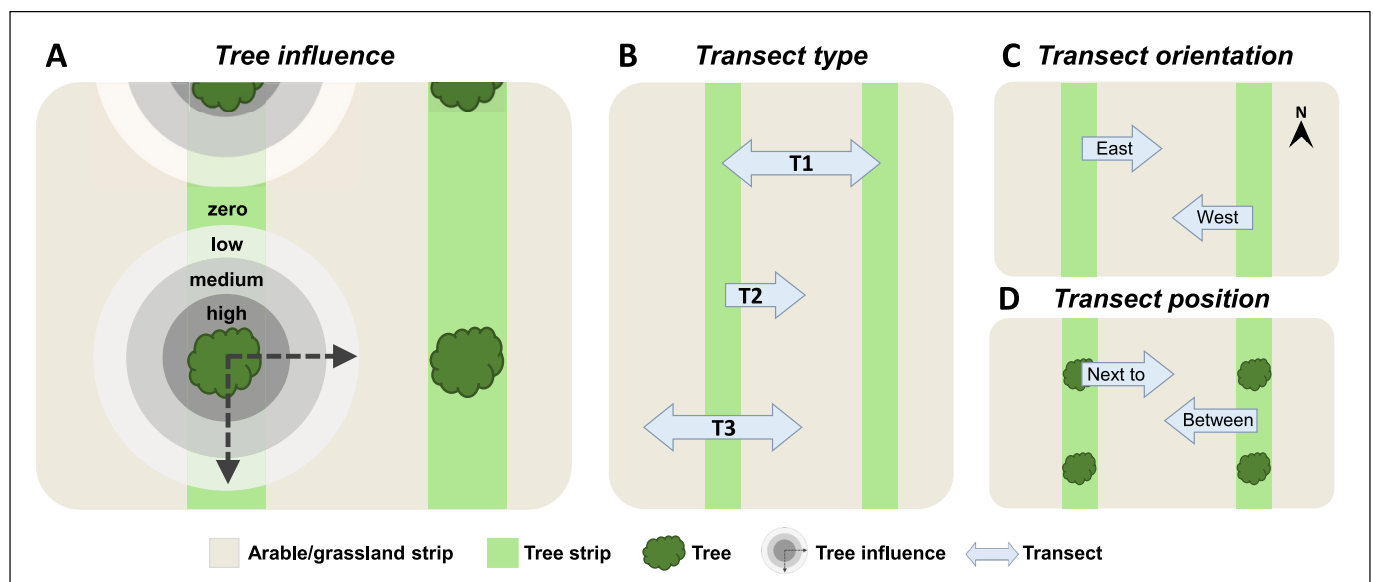


Fig. 2. Schematic illustration of the tree influence and transect soil sampling characteristics in an alley cropping system. A) The tree influence reaches several meters into the tree and arable strips resulting in different levels of intensity (high, medium, low and zero). B) Transects can expand between two tree strips (T1), from one side of the tree strip to the center of the adjacent arable strip (T2) or to both sides of the tree strip to the adjacent arable strip centers (T3). C) Transect orientation to two sides of the tree strips. D) Transect position next to a tree or in between two trees.

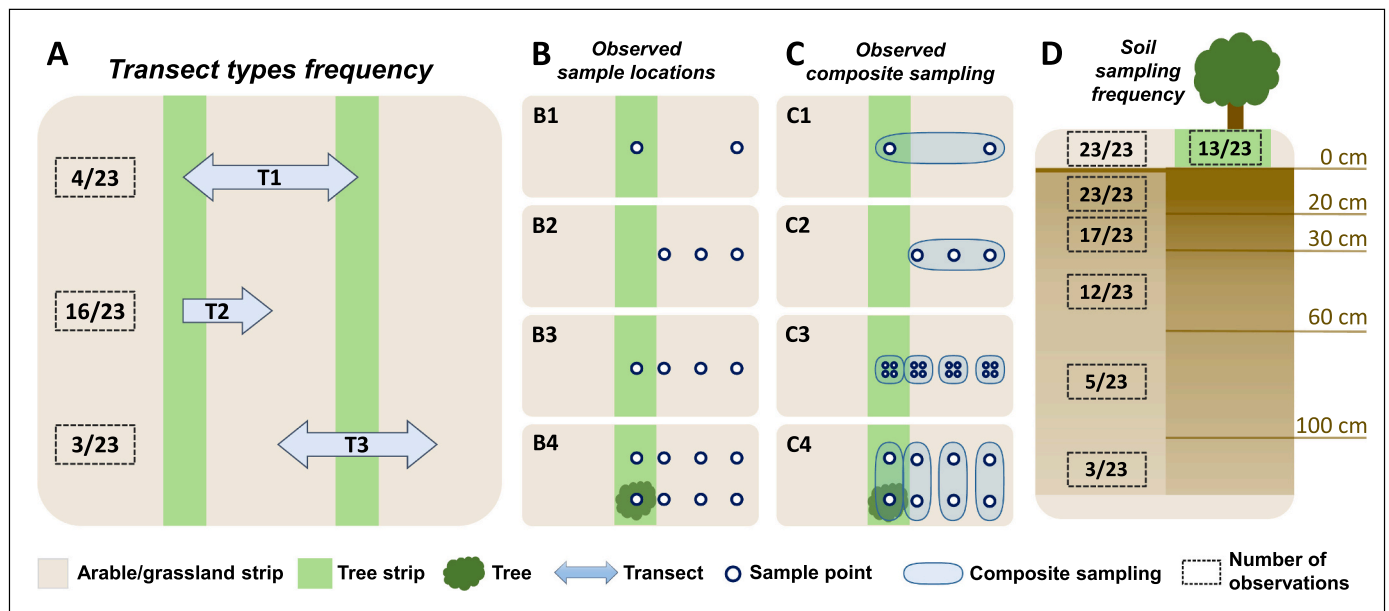
### 3.4. Tree strip sampling bias

The area of a tree strip is heterogeneous and characterized by different levels of tree influence. For instance, over- or underestimation of the tree influence might occur if soil sampling takes place only next to a tree or only in between two trees that are spaced several meters apart, respectively. Furthermore, taking samples only in the center or at the edges of a tree strip can result in a tree strip sampling bias. Ten out of the 23 transect designs did not take soil samples in the tree strip at all, which appears to be the biggest bias observed in this study. The majority of these (nine transect designs) took only one sample per transect, which

can result in the tree strip sampling biases described before. More than one sample per transect (2 and 4 samples) was taken by only four studies and included sampling next to a tree, in between two trees and at other locations (Cardinael et al., 2015, 2017, 2019; D'Hervilly et al., 2020).

### 3.5. Sampling depth bias

Trees in ACS often have deep root systems (Mulia and Dupraz, 2006), which result in carbon transfer into the subsoil, among others. Therefore, soil sampling of at least 1 m depth is recommended (Nair, 2012) to avoid an underestimation of the tree influence. However, in 11 transect



**Fig. 3.** Schematic illustration of the observed transect soil sampling approaches. A) Frequency of observed transect types: T1) in between two tree strips, T2) to one side of the tree strip into the arable strip and T3) to both sides of the tree strip into the arable strips. B) Observed exemplary sample locations with sample points in the tree and arable strip (B1, B3 and B4) or arable strip only (B2). C) Observed exemplary composite sampling approaches: One composite sample per transect (C1 and C2) or four composite samples per transect, each consisting of several sub-samples taken around one sample point (C4) or in two different neighboring transects (C4). D) Frequency of sampling in the tree- and arable strips and in different soil depths (0–20, 20–30, 30–60, 60–100 and below 100 cm). Numbers refer to the identified transect designs out of the total 23 transect designs.

designs no samples were taken below a soil depth of 30 cm. This results in a sampling depth bias for the subsoil (Fig. 3D). Only three studies analyzed below 100 cm with Upson and Burgess (2013) sampling down to 1.5 m and Cardinael et al. (2015) and Gao et al. (2020) down to 2 m.

### 3.6. Weighting for tree- and arable strip area bias

In temperate ACS the tree strip usually covers less area compared to the arable strip. Therefore, when calculating SOC stocks or sequestration rates for the whole ACS, it is important to consider weighting for the tree and arable strip area. Failure to do so can lead to an overestimation of the tree influence. This might have been the case in various studies, because only three transect designs mentioned weighting according to the relative surface areas of the tree and arable strips (Cardinael et al., 2015, 2017; Seitz et al., 2017). Weighting for different areas within the arable strips (e.g. shares of 1 m distance to the tree strip and arable strip center) was not found in any of the studies.

Another bias with regard to weighting for the tree and arable strip area can occur during composite sampling, when sub-samples are taken in the tree and arable strip and combined to a single sample for analysis without weighting for the areas. This approach was used in two transect designs (Fig. 3 C1 and C2), while ten other transect designs used different approaches for their composite samples.

### 3.7. Discussion of analyzed biases

The literature evaluation of SOC analyses in temperate ACS revealed a wide variety of soil sampling approaches. In total six potential biases were identified that can lead to an over- or underestimation of the tree influence in ACS during transect soil sampling with regard to (1) transect orientation, (2) transect position, (3) arable strip sampling, (4) tree strip sampling, (5) sampling depth, and (6) weighting for tree- and arable strip area for quantification of the whole ACS. In each of the 23 transect designs at least one of the six potential biases was found.

We consider the lack of sampling the tree strips in nearly half of the transect designs as the most significant bias observed. This is

problematic for two reasons: Firstly, agroforestry systems are defined as the combination of trees and arable crops or grassland (Nair, 1993), so ignoring the tree strip means ignoring a crucial component of the system. Secondly, studies by D'Hervilly et al. (2020) and Seitz et al. (2017), among many others, have documented higher SOC stocks in the tree strip compared to the arable strip. Therefore, meta-analyses that include (Mayer et al., 2022) or exclusively use (Ivezić et al., 2022) studies that do not sample the tree strip may result in a significant underestimation of SOC for the whole ACS. Another issue we identified was that the majority of studies took only one sample per transect in the tree strip, without considering the potential heterogeneity within the tree strip. Although Cardinael et al. (2017) did not observe a significant difference in the SOC concentration next to a tree or in between two trees, this may vary depending on factors such as tree age, tree spacing and tree strip management.

The bias in arable strip sampling was found to be less prevalent than in tree strip sampling, but was still present due to a high number of transect designs that only took one or two samples per transect. Additionally, many studies were excluded from data extraction because soil samples were only taken at the center of the arable strip (e.g., Clivot et al., 2020) or around a tree (e.g., Wotherspoon et al., 2014). Depending on the research focus of individual studies, it may be sufficient to take soil samples only at these locations. However, we suggest that such studies should not be used for meta-analyses analyzing the SOC stock or sequestration rates of agroforestry systems.

The presence of a bias with regard to transect orientation or position was shown for some studies. However, it was difficult to draw an overall conclusion on the prevalence of both biases, because the respective information was not provided in many studies (for a complete list of provided descriptions see Supplementary Table 2). This highlights the importance of providing a complete description of the ACS design and the soil sampling approach for comprehensibility and reproducibility, also emphasized by other researchers, including Nair (2012), Feliciano et al. (2018) and Cardinael et al. (2018a).

A lack of communication applies also to the bias related to weighting for the tree and arable strip areas. Only three of the 23 transect designs

gave information on the area shares and mentioned weighting for these. No information on this possible bias can be found in the literature, to the best of our knowledge. However, considering the gradients of tree influence that exist in the tree and arable strips (Cardinael et al., 2018b), weighting for the different areas is necessary and may even be relevant for different tree distance areas within the arable strip.

The bias in soil sampling depth, with a lack of subsoil sampling in ACS, was already documented by Mayer et al. (2022) in their meta-analysis. Furthermore, other researchers highlighted the importance of sampling the subsoil for carbon analysis in agroforestry systems (e.g., Nair, 2012; Upson and Burgess, 2013) and other agricultural land-use systems (e.g., Krauss et al., 2022; Wiesmeier et al., 2012).

In conclusion, the literature evaluation highlights the importance of standardizing soil sampling in ACS to mitigate biases of over- or underestimation of the tree influence. The transect soil sampling approach appears to be a promising option, as documented in a series of studies by Cardinael and colleagues in France. Their initial use of a grid sampling design did not account for tree distances (Cardinael et al., 2015). This was replaced by a transect sampling design that considered different positions in the tree and arable strip (Cardinael et al., 2017), and later refined with a significant reduction in the number of samples (Cardinael et al., 2019).

## 4. A proposed guideline for standardized transect sampling

### 4.1. Guideline framework

Based on our literature evaluation and expert discussions, we propose a standardized guideline for transect soil sampling for the analysis of SOC in temperate ACS. This guideline provides recommendations for transect orientation and positioning, the selection of appropriate sample locations in the tree and arable strips, two options for composite sampling, depth distribution for soil sampling down to one meter, and documentation for the ACS and sampling design. It offers the minimum requirements for conducting a comprehensive analysis of an entire ACS and is applicable to other soil analyses (e.g., bacteria and fungi (Beule et al., 2020), earthworms (Cardinael et al., 2019), fine roots and arbuscular mycorrhizal fungi (Battie Laclau et al., 2020)) and ecological studies (e.g., macro-invertebrates (D'Hervilly et al., 2020), crop diseases and mycotoxin accumulation (Beule et al., 2019), crop yield (Swieter et al., 2019)) within ACS where transect sampling is already widely utilized, but lacks a standardized design. Utilizing the same standardized transect sampling design for different parameters allows for a more comprehensive analysis of ACS, as demonstrated by Veldkamp et al. (2023) in their assessment of multifunctionality in ACS. It is important to note, that this proposed guideline does not cover the use of different tools for sampling, analytical methods, calculation approaches and statistical analyses. For the latter, see Golicz et al. (2023), which focuses on the application of multilevel statistical models for the analysis of agroforestry transect sampling data.

### 4.2. Alley cropping system description

To allow comparisons of studies and different ACS, detailed descriptions of the studied systems are mandatory (Feliciano et al., 2018; Nair, 2012). They include information on the experimental site, such as soil type, soil texture, climatic conditions, exposition of the field, the ACS design, and tree and arable strip management. Furthermore, the ACS area and the shares of the tree and arable strips should be documented. A graphic representation of the ACS and the transect soil sampling approach is helpful and should contain strip widths, tree strip orientation, tree spacing and sample locations inter alia. Exemplary templates for description are provided in the Supplementary Checklist T1 and F1.

### 4.3. Transect orientation

Transect type T3 with at least four replicate transects per ACS is recommended (Fig. 4A, see also Fig. 2B for transect types), which is in line with the transect designs from the literature evaluation (2–9 replicate transects with a median and mean of 4). More transects should be used whenever possible, keeping in mind that at least five replicates are necessary for accurate data distribution analysis using boxplots and even more replicates are recommended to increase the reliability of the results (for sample size determination see also Stamps and Linit (1999)). If transect type T2 is used, it is important to consider that the sum of the transects used includes equal shares of both orientations of the tree strip.

### 4.4. Transect position

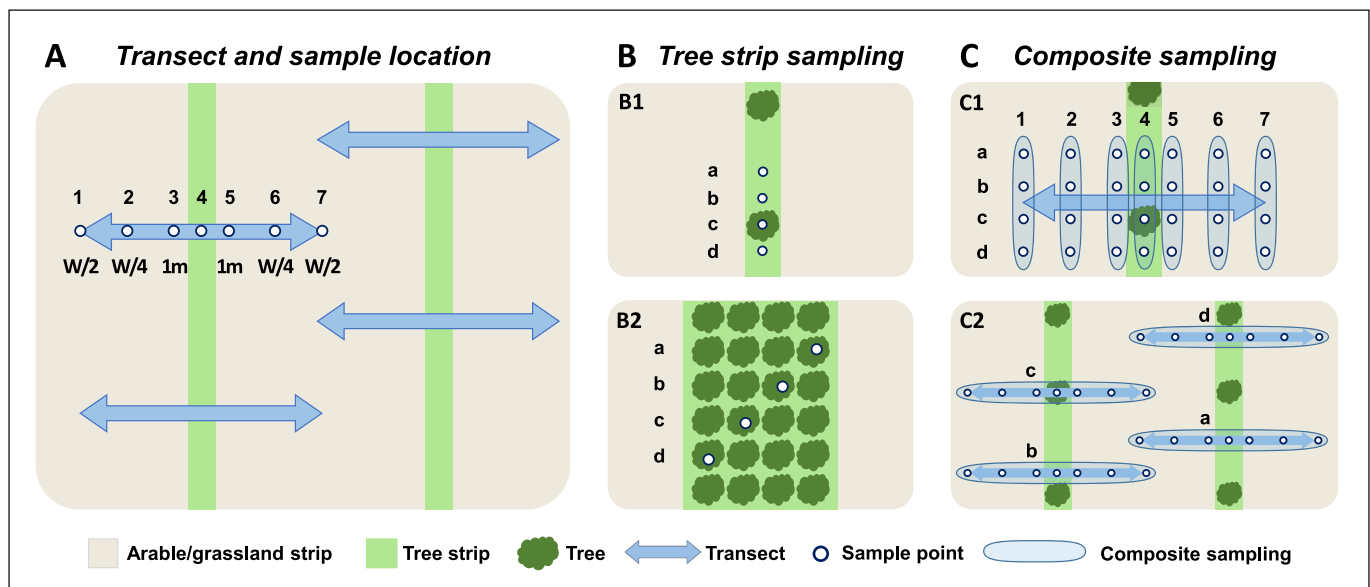
To account for tree strip heterogeneity with regard to tree spacing and tree strip width, we recommend considering high, medium, low and zero levels of tree influence when selecting transect positions. Possible starting points of transects are illustrated in Fig. 4 B1. The above ground levels of tree influence can be determined by tree height and canopy size measurements or tree litter fall analysis in the tree- and arable strips prior to the experiment. Furthermore, geographic information and data such as soil maps, land use history and zones of special concern (e.g. driving lanes, overlaps of different agricultural machinery, former paths or water logging) should be considered for positioning the transects. We recommend that replicated transects are positioned systematically, rather than randomly to characterize an ACS, especially in heterogeneous landscapes, in complex ACS designs, and when only a small number of transects can be used owing to limited labor and financial resources (Stamps and Linit, 1999).

### 4.5. Arable strip sampling

In total a minimum of seven samples within one transect is recommended (Fig. 4A). One sample is taken in the tree strip while the others are taken in the arable strips in about 1 m distance to the tree strip, at one quarter and at half width of the arable strip. The sample taken at 1 m distance to the tree strip corresponds to the most commonly used distance identified in the literature evaluation. It covers the high to medium level of tree influence, whereas the two other samples represent the low and zero levels of tree influence. For the latter two sampling points, no fixed distances are recommended to allow for adaptation to different arable strip widths in different ACS (Cardinael et al., 2017, 2019; D'Hervilly et al., 2020). A higher resolution of sampling points within the transects may have advantages. However, Cardinael et al. (2017) did not observe differences in SOC for samples taken in 2 and 3 m distance from the tree strip in the arable strip of six ACS analyzed.

### 4.6. Tree strip sampling

One sample per transect is taken in the tree strip, resulting in four tree strip samples for the whole ACS. To account for heterogeneity within the tree strips, the four samples may be taken from different sections with varying tree spacing or multiple tree rows. When trees are spaced several meters apart, we recommend taking soil samples near a tree, at a quarter distance to the neighboring tree, and at half distance to the neighboring tree (Fig. 4 B1). This allows to capture high, medium and low or zero levels of tree influence, and is in accordance with the approach applied by Cardinael et al. (2017). When tree strips have multiple rows of trees, we recommend taking soil samples at locations in the center of the strip and towards the edges (Fig. 4 B2). This allows to cover possible effects due to differences in tree growth in the center and at the edges of the tree strips, which was observed by Lamerre et al. (2015) for poplars in a short-rotation coppice ACS. Both tree strip sampling approaches can detect potential differences in the tree influence and understory vegetation cover, thereby representing the whole



**Fig. 4.** Schematic illustration of the proposed transect soil sampling approach. A) Transect and sample locations: Four transects placed at representative locations in the ACS and each consisting of seven sample points. One sample point in the tree strip and three sample points in the arable strip on both sides, with distances to the edge of the tree strip of 1 m, a quarter ( $W/4$ ), and half ( $W/2$ ) of the arable strip width. B) Tree strip sampling (two examples): B1) Tree strip with tree spacing of several meters: Sample points are next to a tree (c), between two trees (a) and at a quarter distance to the neighboring tree to both sides (b and d). B2) Tree strip with multiple tree rows: Sample points are in the strip center (b and c) and close to the edges (a and d). C) Composite sampling (two examples): C1) Each of the four transects consists of four sub-transects (a-d) located around one key tree. The four sub-samples (e.g., 1a-1d) of each transect are combined ( $n = 7$  per transect). C2) The four transects are distributed over the whole ACS and positioned next to a tree (c), in between two trees (a) and at a quarter distance between two trees (b and d). Samples are combined transect wise under consideration of weighting for the tree and arable strip areas ( $n = 4$  per system).

tree strip. Accounting for tree strip heterogeneity can lead to a high sample number resulting in high costs for sampling and analyses. These costs can be reduced by composite sampling.

#### 4.7. Composite sampling

The composite sampling approach used most frequently in the studies of the literature evaluation involved the collection of multiple sub-samples in close vicinity to a sampling point and their combination to one composite sample for analysis (Fig. 3 C3). With this approach small scale soil heterogeneity can be addressed (Gojdt et al., 2009). Composite sampling can also be used to account for the tree strip and overall system heterogeneity as shown by Oelbermann et al. (2006) in their study of a Costa Rican ACS. Here, sub-samples were collected in two neighboring transects that were positioned next to a tree and in between two trees (Fig. 3C4). In this guideline, we suggest two options for composite sampling. In option one, each of the four transects consists of four sub-transects that are located around a tree at the positions next to the tree, at half distance and at both quarter distances to the neighboring tree (Fig. 4 C1). The samples of the four sub-transects can be combined according to their seven locations resulting in seven samples per transect for analysis. In option two, the four transects are positioned at the same four locations described for option one, but scattered around the whole ACS (Fig. 4 C2). All seven samples from one transect can be combined to one composite sample, if the areal proportions of the tree and arable strips are considered. This requires quantifying the soil water content of each sub-sample before combining them to generate the composite sample. In addition, the bulk density must be taken into account when generating composite samples, which is particularly important for option two. There can be a large variation in the bulk density of samples from the tree strip compared to the arable strip, which would argue against the generation of these composite samples.

#### 4.8. Sampling frequency

Soil monitoring over several decades may be necessary to evaluate long-term influences, considering that agroforestry systems develop over many years and land-use history effects can persist over long periods (Jones et al., 2016). Furthermore, as Kim et al. (2016) and Feliciano et al. (2018) showed, C sequestration is higher in younger and lower in older agroforestry systems. Ideally, baseline data from the time of establishment of the system is collected using a transect sampling approach. Repetitive sampling to monitor stock changes should be conducted at the same time of the year and as closely as possible to previous sampling points. Geo-referencing of sampling locations is recommended, as SOC stocks can vary significantly even on a small scale (Poeplau et al., 2022). Because changes in SOC stocks are slow (Wiesmeier et al., 2020) sampling intervals of five years seem sufficient. If comparison with a non-agroforestry control site is planned, baseline data for this site is necessary as well to document differences from the beginning. In the absence of a suitable non-agroforestry control, researchers may decide to use the arable strip center as a control. However, this is only recommendable in ACS with small trees and/or wide arable strips (see also the discussion from Peichel et al. (2006)).

#### 4.9. Sampling depth

Soil sampling below the topsoil horizon is recommended for the evaluation of agricultural management practices (Wiesmeier et al., 2012) and is of importance for agroforestry systems, since perennial plants are expected to have a deep root system (Mulia and Dupraz, 2006). Conducted in over three thousand sampling sites, the national agricultural soil inventory (Jacobs et al., 2018) has provided a standard for Germany. The soil depth distributions used are 0–10, 10–30, 30–50, 50–70 and 70–100 cm for arable and grassland. In order to account for field and soil depth heterogeneity and to quantify SOC stocks accurately, soil bulk density has to be determined in parallel (Walter et al., 2016).

## 5. Conclusions and outlook

In our literature evaluation, we identified significant variation and potential biases in transect soil sampling approaches for SOC employed in ACS. Many studies failed to account for the heterogeneity resulting from trees in ACS, which may have resulted in over- or underestimation of the tree influence on the system as a whole. Additionally, inadequate descriptions of experimental designs impede comparisons between studies, making it challenging to draw definitive conclusions. This highlights the need for a standardized transect sampling approach for ACS, along with clear documentation of the ACS design. The proposed guideline aims to contribute to improved field study design and description, and the selection of suitable studies for meta-analyses.

## CRediT authorship contribution statement

**Eva-Maria L. Minarsch:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Philip Schierning:** Formal analysis, Methodology, Visualization, Writing – review & editing. **Florian Wichern:** Methodology, Supervision, Writing – review & editing. **Andreas Gattinger:** Funding acquisition, Methodology, Supervision, Writing – review & editing. **Philipp Weckenbrock:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## Acknowledgements

The authors would like to thank Dr. Christian Böhm, Thomas Middelanis and Dr. Leonie Göbel as co-authors, and Dr. Rico Hübner and Prof. Norbert Lamersdorf as reviewers, for their work on the development of the DeFAF guideline for soil monitoring in ACS which is the basis of this study. E-MLM, AG and PW would like to acknowledge funding of the projects “Agroforstsysteme Hessen” (VII 5-80e04-09-04) and “Agroforstsysteme Hessen II” (895-0736-23-511-8-028) through the Hessische Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz as part of the Hessische Ökoaktionsplan 2020-2025.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geodrs.2024.e00757>.

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