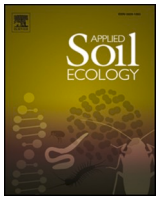
Applied Soil Ecology 195 (2024) 105277

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil



Main soil microbial groups assessed by phospholipid fatty acid analysis of temperate alley agroforestry systems on crop- and grassland

Katharina Giray [a], Callum Banfield [b], Hans-Peter Piepho [c], Rainer Georg Joergensen [a,*], Michaela Dippold [b], Christine Wachendorf [a,1]

[a] Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany
[b] Geo-Biosphere Interactions, Department of Geosciences, University of Tübingen, Schnarrenbergstr. 94-96, 72076 Tübingen, Germany
[c] Biostatistics Unit, Institute of Crop Science, University of Hohenheim, Fruwirthstr. 23, 70593 Stuttgart, Germany

ARTICLE INFO

Keywords:
Poplar
Willow
Microbial groups
Microbial biomass
Mycorrhizal fungi
Saprotrophic fungi

ABSTRACT

The phospholipid fatty acid (PLFA) composition of soils was analysed at three poplar-based silvo-arable systems and at one willow-based silvo-grassland alley agroforestry system in Central Germany. The objective was to analyse tree row effects on the PLFA composition of main fungal and main bacterial groups. The fungal groups were BAM (Basidiomycota + Ascomycota + Mucoromycota) and AMF (Arbuscular Mycorrhizal Fungi). The bacterial groups were Gram-negative, Firmicutes, and Actinobacteria. The total PLFA content varied between 53 and 170 nmol g^{-1} soil. Total PLFA and microbial biomass carbon (MBC) showed a strong linear relationship, which resulted in a mean MBC/total PLFA ratio of 4.2 μg $nmol^{-1}$. AMF contributed on average 4 mol% and the fungal BAM group 10 % to total PLFA. Gram-negative bacteria contributed on average 37 mol%, Firmicutes 23 mol%, and Actinobacteria 6 mol% to total PLFA. The presence of poplar or willow trees increased the mean total PLFA content in comparison with the alleyways by 30 %. Especially the mean contribution of fungal PLFA to total PLFA showed a significant +7.0 mol% increase in the tree row compared with the alleyways, exclusively caused by the BAM group (+7.6 mol%), whereas the contribution of the AMF PLFA linearly decreased from the middle of the alleyway to the tree row at all sites. Within the alleyways, the Gram-negative/Firmicutes PLFA ratio showed a significant decline from the 1 m up to the 24 m distance samples at sites Dornburg and Forst. Despite a decrease of AMF in tree rows, agroforestry tree rows led to a rapid increase in fungi, most likely due to the promotion of ecto-mycorrhizal fungi.

1. Introduction

Agroforestry systems have been established in Central Europe over the last decades to provide biomass as an energy source, to improve biodiversity, to protect against soil erosion, and to increase carbon (C) sequestration in soil organic matter (Lawson et al., 2019; Udawatta et al., 2019). Three poplar-based silvo-arable alley cropping and one willow based silvo-grassland alley agroforestry systems were installed on different soil types in Central Germany between 2008 and 2011 (Beuschel et al., 2019, 2020). The four sites markedly differ in their soil texture and soil organic matter content (Table 1). Soil properties and especially different microbial characteristics have been intensively investigated in these agroforestry systems, because soil microorganisms are the key drivers of nutrient cycling.

Key indices were microbial biomass (MB), potential enzyme activities, functional diversity, microbial necromass formation (Beuschel et al., 2019, 2020) and microbial community composition (Beule and Karlovsky, 2021; Beule et al., 2020, 2021), using DNA extraction, amplicon sequencing, and quantitative real-time polymerase chain reaction (*q*PCR). In the tree rows, significant increases in soil organic C (SOC), fungal and bacterial gene copies, microbial necromass C, MBC, MBN, fungal ergosterol, and in the fungal C/bacterial necromass C ratio have been observed after 5 to 8 experimental years, i.e., after a relatively short time of land-use change. However, most changes only occurred at 0–5 cm depth and not at 5–20 cm depth (Beuschel et al., 2019, 2020), which is consistent with other studies under temperate climatic conditions (Mungai et al., 2006; Sun et al., 2018; Battie-Laclau et al., 2020).

However, information on arbuscular mycorrhizal fungi (AMF) is still

* Corresponding author.
E-mail address: joerge@uni-kassel.de (R.G. Joergensen).
[1] Current address: Soil Science, University of Kassel, Germany.

https://doi.org/10.1016/j.apsoil.2024.105277
Received 27 September 2023; Received in revised form 4 January 2024; Accepted 5 January 2024
Available online 12 January 2024

Table 1
Site characteristics and management features at the four agroforestry sites of the current study (Beule et al., 2020; Beuschel et al., 2019, 2020; Zhou et al., 2022).

	Dornburg	Forst	Wendhausen	Reiffenhausen
Latitude	51°00′40”	51°47′11”	52°20′00”	51° 39′83”
Longitude	11°38′40”	14°38′05”	10°37′55”	9° 98′75”
ASL (m)	289	87	82	325
MAP (mm for 1981–2010)	612	568	637	649
MAT (°C for 1981–2010)	8.9	9.6	9.5	9.1
Start of experiment (a)	2007	2010	2008	2011
Soil type (IUSS Working Group WRB, 2022)	Calcaric Phaeozem	Gleyic Cambisol	Vertic Cambisol	Eutric Cambisol
Tree row	Poplar	Poplar	Poplar	Willow
Tree row harvest (month/a)	01/2015	02/2015	01/2014	02/2015
Alleyway crop at sampling	Oilseed rape	Maize	Winter wheat	Grass/clover
Soil sampling (month/a)	10/2015	10/2015	11/2015	10/2016
Sand (%, 0–20 cm)	4	64	15	33
Silt (%, 0–20 cm)	70	27	36	47
Clay (%, 0–20 cm)	26	9	49	20
Soil pH-H_2O (0–20 cm)	6.9	7.4	7.7	6.2
SOC tree row (%, 0–5 cm)	2.15	1.25	2.97	1.66
SOC alleyway (%, 0–5 cm)	1.60	1.12	2.63	1.67

ASL = above sea level; MAP = mean annual precipitation; MAT = mean annual temperature.

lacking at these four alley agroforestry systems, despite the importance of this fungal phylum for plant nutrition (van der Heijden et al., 2015), rhizodeposition (Zhou et al., 2020; Bicharanloo et al., 2022), and soil aggregation (Agnihotri et al., 2021). This lack of knowledge is especially unfortunate, due to the uncertain estimates of AMF contribution to the soil microbial biomass, ranging from <0.5 % (Hartmann et al., 2015) to >30 % (Faust et al., 2017). Studies that analysed the AMF-specific phospholipid fatty acid (PLFA) 16:1ω5 showed that this fungal phylum in particular benefits from agroforestry systems (Lacombe et al., 2009; Unger et al., 2013). Indirect negative effects of willows on AMF have been observed by Becklin et al. (2012), which would be in line with the increased ergosterol content in tree rows (Beuschel et al., 2019, 2020). This suggests an increased contribution of ectomycorrhizal or saprotrophic fungi to the microbial biomass, because AMF do not contain ergosterol (Olsson et al., 2003). However, poplar and willow form a symbiosis with both AMF and ectomycorrhizal fungi (Gherghel et al., 2014; Fortin Faubert et al., 2022). This might be reflected by the increase in the respective fungal PLFA 16:1ω5 (AMF), 18:1ω9 (mainly Mucoromycota), 18:2ω6,3 (mainly Ascomycota and Basidiomycota), and unspecific 18:3ω3,6,9 (Joergensen, 2022).

The objective of the current study was to analyse tree row effects on the PLFA composition of main fungal and main bacterial groups. The approach was to analyse the PLFA composition of three poplar-based silvo-arable sites at Dornburg, Forst, and Wendhausen, as well as one willow based silvo-grassland systems at Reiffenhausen. The PLFA data are additionally compared with MBC and ergosterol data provided by Beuschel et al. (2019, 2020). This approach was chosen to investigate the following two hypotheses: (1) Tree rows shift the PLFA composition to more ecto-mycorrhizal and saprotrophic fungal biomarkers (18:1ω9 and 18:2ω6,9), compared with the alleyways. (2) The AMF marker (16:1ω5) is lower in tree rows than in alleyways.

As three of the four sites have been intensively investigated over recent years, it was also possible to compare the relationships of MBC with total PLFA data and those of ergosterol with fungal PLFA obtained from the same soil samples. This should confirm the reliability of the PLFA approach (Joergensen, 2022), which has been intensively used over the last decade, often without sufficient methodological validation. In addition, PLFA analysis not only provides information on fungi but also on three main bacterial groups, i.e., Gram-negative bacteria, Firmicutes, and Actinobacteria (Joergensen, 2022). For this reason, PLFA data are an important independent control of DNA-based *q*PCR and sequencing techniques (Lewe et al., 2021). Consequently, the current study should also provide evidence of the extent to which membrane- and genome-based approaches give similar information on core groups of soil microbial communities.

2. Materials and methods

2.1. Sites, sampling, and soil analysis

The present study was based on soils from three German arable alley agroforestry systems: Dornburg, Thuringia, Wendhausen, Lower Saxony, and Forst, Brandenburg (Fig. 1, Table 1). Poplar (clone Max 1, *Populus maximowiczi* × *P. nigra*) rows were planted in north-south orientation with 48-m wide alleys in between for crop production (Fig. 1). An additional grassland alley agroforestry system was investigated at Reiffenhausen, south Lower Saxony. Willows (clone Tordis, (*Salix viminalis* × S. *Schwerinii*) × *S. viminalis*) were planted in rows in north-west to south-east orientation with 9-m wide grassland alleyways in between. Each tree strip consisted of four double rows. Spacing between double and single rows was 1.5 and 0.75 m, respectively.

The soil samples were taken once at 0–5 cm depth by René Beuschel with a steel auger (4.2 cm diameter), combining 6 cores to one bulk sample from each specific distance point of each of the four transects (Beuschel et al., 2019, 2020). Sampling was conducted in October 2015 at Dornburg and Wendhausen as well as in November 2015 at Forst (Table 2). At these three sites, the sampling points were in the middle of the tree row in width, after 1 m, 7 m and in the middle of the crop rows at 24 m distance from the tree row (Beuschel et al., 2019). Another sampling was conducted in October 2016 at Reiffenhausen (Beuschel et al., 2020), where the sampling points were in the middle of the tree row in width, after 1 m and in the middle of the grassland at 4.5 m distance from the tree row. Measurement always started after 0.5 m from tree trunks.

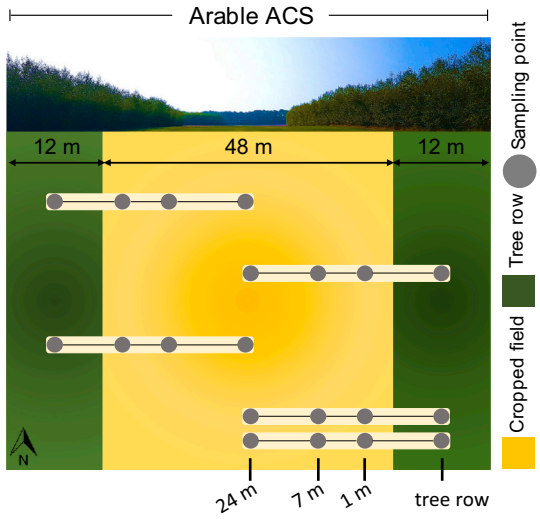


Fig. 1. Experimental design of arable alley cropping systems (ACS), using the example of Dornburg, Germany. Tree rows are shown to the right and left of the crop row. Sampling points are in the middle of the tree rows, and at 1, 7 (4.5 m in the grassland ACS at Reiffenhausen) and 24 m (not in Reiffenhausen) distance from the tree row.

Table 2
Mean of total PLFA contents in soil, mean contribution of total bacteria, Gram(−) bacteria, Firmicutes, Actinobacteria, total fungi, AMF and BAM to total PLFA in soils under the tree rows and the alleyways at the four sites.

Site/position	Total PLFA	Bacteria	Gram(−)	Firmicutes	Actinobacteria	Fungi	AMF	BAM
	(nmol g^{-1} soil)	(mol%)						
Dornburg								
Tree row	128	64.9	38.2	20.8	5.8	17.0	4.2	12.6
Alleyway	86	69.8	34.9	26.2	8.8	11.8	5.0	6.8
Forst								
Tree row	75	58.9	39.0	14.7	5.0	21.0	3.9	16.9
Alleyway	54	64.7	38.1	21.4	5.1	12.0	4.8	7.2
Wendhausen								
Tree row	170	66.5	39.7	20.4	6.5	15.7	4.0	11.8
Alleyway	134	69.3	39.3	24.0	6.0	12.3	4.1	8.2
Reiffenhausen								
Tree row	121	51.7	22.8	24.5	4.5	22.9	3.4	19.5
Alleyway	107	64.4	39.5	21.7	5.1	12.4	3.9	8.5
Probability values								
Site	<0.01	<0.01	<0.01	0.01	<0.01	0.01	0.01	<0.01
Tree vs AW	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Site × tree vs AW	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	NS	<0.01
Distance	<0.01	NS	<0.01	0.01	NS	NS	0.01	NS
Distance × site	<0.01	0.01	0.01	NS	<0.01	NS	NS	NS
CV (± %)	6.9	9.0	9.7	13	12	9.7	9.9	11

CV = Mean coefficient of variation between replicate samples ($n = 4$); AW = alleyway; AMF = arbuscular mycorrhizal fungi; BAM = Basidiomycota + Ascomycota + Mucoromycota.

After transportation from the fields under cold conditions, soil was sieved (mesh size <2 mm) and aliquots immediately stored frozen (−20 °C) until PLFA analysis (Stenberg et al., 1998). SOC, MBC, and ergosterol data were provided by René Beuschel and have already been published (Beuschel et al., 2019, 2020). Total C was determined after combustion using an elemental analyser. MBC was analysed by fumigation extraction (Vance et al., 1987; Wu et al., 1990) and ergosterol after ethanol extraction (Djajakirana et al., 1996), as described in detail by Beuschel et al. (2019).

2.2. Phospholipid fatty acid analysis

PLFA were extracted by a modified version of Frostegård et al. (1991), as described by Dippold and Kuzyakov (2016) and Gunina et al. (2014). PLFA were extracted from 6 g of frozen soil with a solution of methanol, chloroform, and citrate/KOH buffer (pH 4, v/v/v = 1/2/0.8) according to Bligh and Dyer (1959). Purification and separation of the PLFAs was carried out, using a solid phase extraction (SPE). For measuring the specific PLFAs, solved extracts were hydrolyzed and methylated to fatty acid methyl esters (FAMEs). The prepared samples were measured on a gas chromatograph-mass spectrometry system (GC 7820 A, MS 5977B, Agilent Technologies, Waldbronn, Germany) and displayed with the program Mass Hunter, using an external standard.

The sum of Firmicutes-specific iso- and anteiso-branched saturated (i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0) as well as Actinobacteria-specific ester linked 10-methyl branched saturated fatty acids (10Me 16:0 and 10Me 18:0) represented Gram-positive [Gram(+)] bacteria (Zelles, 1997; Barka et al., 2016). The sum of cyclopropyl (cy17:0 and cy19:0) and monounsaturated fatty acids (16:1ω9 and 18:1ω7) represented Gram-negative [Gram(−)] bacteria (Frostegård et al., 1993; Zelles, 1997). PLFA 16:1ω5 represented arbuscular mycorrhizal fungi (AMF), and 18:1ω9, 18:2ω6,9, and 18:3ω3,6,9 saprotrophic and ectomycorrhizal fungi (Olsson et al., 1995; Olsson, 1999; Joergensen and Wichern, 2008). PLFA 14:0, 15:0, 16:0, and 18:0 are common to both bacteria and fungi (Zelles, 1997; Joergensen, 2022) and were only used to obtain total PLFA, which was calculated as the sum of all identified PLFA. The Shannon Diversity Index (H) was calculated according to Zak et al. (1994): $H = -\sum_i p_i ln(p_i)$, where p_i is the specific PLFA relative frequency.

2.3. Statistical analysis

Data are presented in tables and figures as arithmetic means on an oven-dry weight basis (ca. 24 h at 105 °C). Sampling point distance in the alleyways from the tree rows was handled as a quantitative covariate to consider their spatial dependency (Beuschel et al., 2019; Golicz et al., 2023). Lack-of-fit of the linear regression was also tested, modelling site × distance as qualitative effects (Piepho and Edmondson, 2018). In the case of a significant lack of fit, an additional factor was added that separates the tree row from the alleyway, so that the regression was fitted excluding the tree row. Spatial correlations within transects were modelled using an exponential model with nugget. In addition, a random main effect for transects within sites was fitted for all response variables to obtain a full variance-covariance structure. However, the residual maximum likelihood algorithm may converge to a fit that fixes one or several of the variance parameters at the boundary and effectively takes these terms out of the model. This behavior implies an automatic model selection for the variance-covariance structure. Degrees of freedom were calculated using the Kenward-Roger method.

Data were log-transformed if the requirements of normal distribution and homogeneity of variances was not fulfilled. Presented data were back transformed to the original scale. Significant distance effects within the alleyway and interrelationships between the microbial groups were evaluated by linear regressions. If significant distance or distance × site effects were reported, the uneven distance between the sampling points in the alleyways was accounted for by reporting the mean values for the alleyways, using the calculated value of the mean width of the linear regression. All statistical analyses were performed with SAS (Statistical Analysis System, version 9.4, SAS Institute, Cary, NC, USA).

3. Results

3.1. Site effects on PLFA

The total PLFA content varied between 53 (Forst, alleyway) and 170 (Wendhausen, tree row) nmol g^{-1} soil (Table 2). Total PLFA showed a strong positive linear relationship with MBC (Fig. 2a), which resulted in a mean MBC/total PLFA ratio of 4.2 μg $nmol^{-1}$. The PLFA content of the different microbial groups showed strong interrelationships ($P < 0.01$), with correlation coefficients of between 0.55 and 0.93 ($n = 60$). The contents of BAM PLFA and ergosterol showed a strong linear

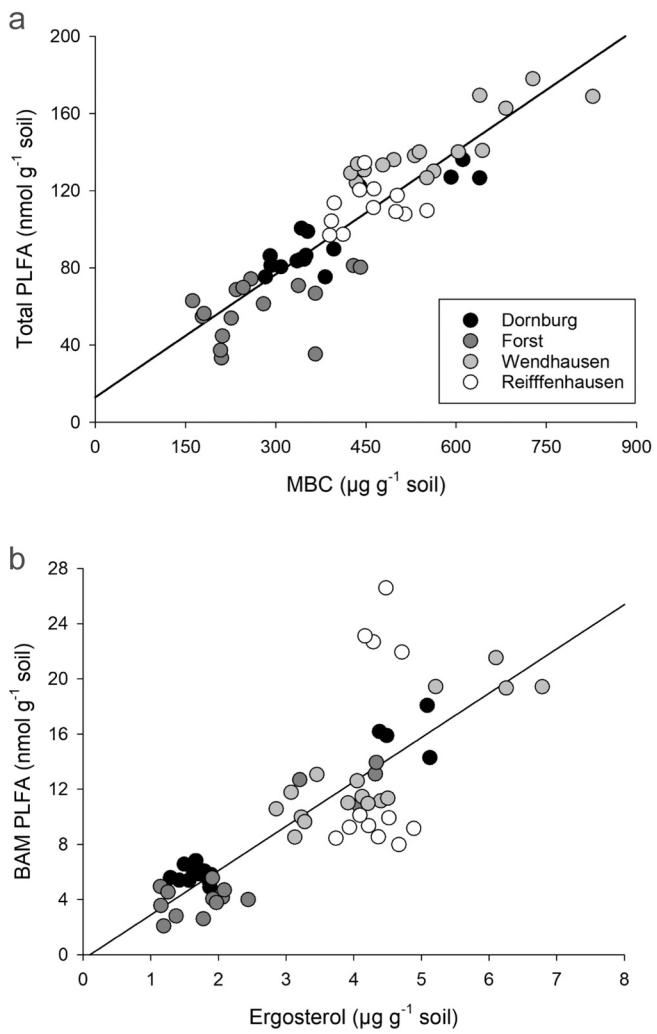


Fig. 2. Linear relationship between (a) MBC and total PLFA (y = 12.969 + 0.212, $r = 0.89$, $P < 0.01$, n = 60) as well as (b) BAM PLFA and ergosterol (y = −0.323 + 3.214, $r = 0.81$, $P < 0.01$, n = 60); MBC and ergosterol data were taken from Beuschel et al. (2019).

relationship (Fig. 2b), which resulted in a mean BAM/ergosterol ratio of 3.1 nmol μg^{-1}. However, considerable deviations from this ratio were observed at the grassland site Reiffenhausen in the tree rows.

The contribution of the different microbial groups to total PLFA in mol% was significantly affected by site × tree versus alleyway interactions (Table 2). Bacteria contributed on average 65 % to total PLFA, Gram(−) bacteria 37 mol%, Firmicutes 23 mol%, and Actinobacteria 6 mol%, whereas AMF provided on average 4 mol% and the BAM group 10 % to total PLFA.

The effect of distance and distance × site was significant for total PLFA, contribution of Gram(−) bacteria and BAM to total PLFA. The mean ratio of Gram(−)/Gram(+) PLFA was 1.34 (results not shown) and that of Firmicutes/Actinobacteria PLFA 3.9 (Table 3). The ratio of Gram (−)/Firmicutes PFLA varied around an average of 1.72 and significantly declined from the 1 m to the 24 m distance samples at Dornburg and Forst and from 1 m to 4.5 m at Reiffenhausen but not at Wendhausen (Fig. 3). The Shannon diversity index of the microbial PLFA distribution varied around 2.57 and was markedly lower in the alleyway at the grassland site Reiffenhausen (Table 3).

3.2. Tree effects on PLFA

The presence of trees increased the mean total PLFA content in

Table 3
Mean PLFA ratios of Firmicutes to Actinobacteria, Gram(−) to Firmicutes, BAM to AMF, and fungi to bacteria as well as the Shannon diversity index in soils under the tree rows and the alleyways at the four sites.

Site/position	Firmicutes/ Actinobacteria	Gram(−)/ Firmicutes	BAM/ AMF	Fungi/ Bacteria	Shannon index
Dornburg					
Tree row	3.5	1.8	3.0	0.26	2.67
Alleyway	3.0	1.4	1.4	0.17	2.71
Forst					
Tree row	3.6	2.7	4.3	0.35	2.55
Alleyway	4.2	1.9	1.5	0.19	2.61
Wendhausen					
Tree row	3.0	2.0	2.9	0.24	2.67
Alleyway	4.1	1.6	2.0	0.18	2.71
Reiffenhausen					
Tree row	5.6	0.9	5.8	0.44	2.65
Alleyway	4.1	1.9	2.2	0.19	2.20
Probability values					
Site	0.01	<0.01	<0.01	<0.01	<0.01
Tree vs AW	NS	0.02	<0.01	<0.01	<0.01
Site × tree vs AW	<0.01	<0.01	<0.01	<0.01	<0.01
Distance	0.02	0.01	NS	NS	<0.01
Distance × site	<0.01	0.03	NS	NS	<0.01
CV (± %)	16	13	12	9.0	1.9

CV = Mean coefficient of variation between replicate samples (n = 4); AW = alleyway; BAM = Basidiomycota + Ascomycota + Mucoromycota; AMF = arbuscular mycorrhizal fungi.

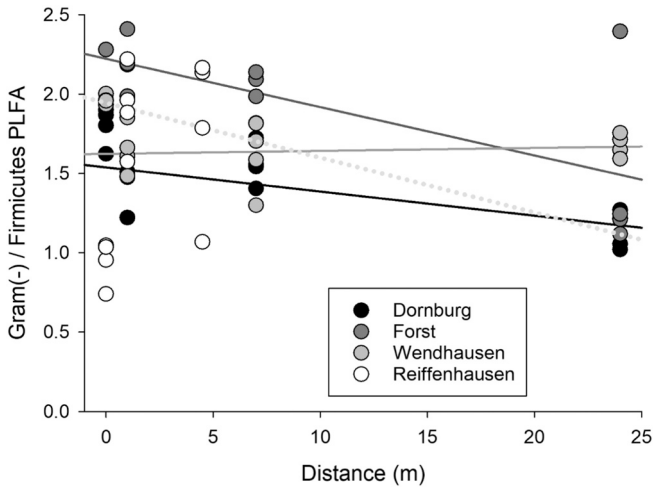


Fig. 3. Measured values in tree rows and alleyways and modelled linear relationships of Gram(−)/Firmicutes PLFA and distance within the alleyways for the sites Dornburg (y = 1.5388*** - 0.0152* x), Forst (y = 2.2237*** - 0.0305*** x), Wendhausen (y = 1.6245*** + 0.0018 x), and Reiffenhausen (y = 1.9456*** - 0.0345 x), with significant interaction of distance and site; * $P < 0.05$, *** $P < 0.001$.

comparison with the alleyways by 30 % (Table 2). This was especially true for the mean contribution of fungal PLFA to total PLFA, which showed a significant +7.0 mol% increase in the tree rows in comparison with alleyways, exclusively caused by the BAM group (+7.6 mol%). At all sites AMF specific PLFA 16:1ω5 exhibited lower values under the tree rows. The mean BAM/AMF PLFA ratio showed a strong decline from 4.0 in the tree rows to 1.8 in the alleyways (Table 3). Consequently, the ratio of fungal (AMF + BAM) to bacterial PLFA exhibited a less pronounced decrease from 0.33 in the tree rows to 0.18 in the alleyways. Conversely to fungal PLFA, the mean contribution of bacterial PLFA significantly increased from the tree rows to the alleyways. This was mainly caused by the low contribution of Gram(−) bacteria to total PLFA at the site

Reiffenhausen with willow trees and by the low contribution of Firmicutes at the Forst site.

4. Discussion

4.1. General agroforestry effects on PLFA

The growth of poplar and willow trees in rows for a relatively short period of between 5 and 8 years had strong effects not only on the soil microbial biomass but also on the contribution of different microbial groups to the microbial biomass of the four alley agroforestry sites. The increasing effects of tree row implementation on total PLFA and the contribution of BAM and total fungi to PLFA were much stronger than those on SOC (Tables 1 and 2). This indicates that microorganisms responded faster than the total sequestered SOC pool to changes in land-use management (Powlson et al., 1987), like the current tree row implementation. Strong site effects were observed on PLFA content in nmol g^{-1} soil, probably caused by differences in soil and climatic conditions but also by the different tree species planted and by the different periods of alley agroforestry management. In contrast to the PLFA contents, the site effects on PLFA composition in mol% were considerably smaller, despite these differences. This suggests that stable relationships exist between the different microbial groups in arable and most likely also in grassland ecosystems.

Total PLFA showed a tight significant linear relationship with MBC, suggesting that it may be used as a valid biomass indicator. This indicates a relatively low variation in average cell size across all sites, as this would change the surface/volume (= cytosol) ratio, although differences in the C supply by tree rows, crops, and grassland are likely. The ratio of MBC to PLFA has been shown to depend on the C supply and, consequently, on the cell size: the smaller the cell, the higher the contribution of cell membrane components to the microbial biomass (Meyer et al., 2019, 2021).

The current mean MBC/total PLFA ratio was markedly below the mean ratios of 5.8 (Joergensen and Emmerling, 2006), 6.0 (Joergensen and Potthoff, 2005), and 7.1 (Faust et al., 2017) presented in previous publications. However, the current MBC/total PLFA ratio is still within the range of 2.9 to 13.8 μg $nmol^{-1}$ given by Joergensen and Emmerling (2006). The reasons for this range are not really known, hampering the interpretation of PLFA data if occurring in repeated samplings within an experiment (Murugan et al., 2021). Differences in the extraction conditions are one reason, other reasons are the separation and detection procedures, leading to different numbers and amounts of PLFA within a soil sample. An important source of variation is also the soil moisture during PLFA extraction (Zelles, 1999), whereas the soil type has apparently only a minor effect. In contrast to the substrate induced respiration (SIR) method, the conversion of total PLFA to MBC has not gained acceptance, although it would be an independent proof for the reliability of this approach.

4.2. Agroforestry effects on BAM fungi

The most striking tree row effect of the current study was the strong increase in fungal PLFA (18:1ω9c + 18:2ω6,9 + 18:3ω3,6,9) and in ergosterol of the BAM group. This increase was due to the increased presence of ectomycorrhizal Basidiomycota (Beule et al., 2021). This result is in line with Beule et al. (2020), who observed an increased number of fungal gene copies in the tree rows in comparison with the alleyways of the three poplar sites. In particular, the abundance of Ascomycota and Basidiomycota was higher in the tree rows at Forst and Wendhausen than in the respective alleyways (Beule et al., 2020, 2021). Tree rows increased not only the population size but also species richness of soil fungi (Beule et al., 2021). Fungal amplicon sequence variants were assigned to 14 fungal phyla (Beule et al., 2021) dominated by Ascomycota (66 %), Basidiomycota (26 %), and Mucoromycota (6 %). The low contribution of Mucoromycota contrasts the high concentrations of oleic acid 18:1ω9c, with an average of 7.6 mol% in the current study. Oleic acid is the dominating PLFA of Mucoromycota (Joergensen and Wichern, 2008).

At Dornburg, Basidiomycota were up to 96 times more abundant, comparing the tree row with the 7-m and 24-m distance samples (Beule et al., 2020). A considerable variation in fungal gene copies was observed between the three poplar sites. This contrasts Meyer et al. (2021), who found a roughly constant mean ratio of 71×10^7 fungal gene copies to μg^{-1} ergosterol in soils.

The three BAM PLFA showed a strong correlation with ergosterol, which mainly occurs in Ascomycota and Basidiomycota and to a largely unknown extent in Mucoromycota (Joergensen, 2022). Only at the grassland site Reiffenhausen was the relationship between the BAM PLFA and ergosterol rather weak. This is presumably due to the presence of the unspecific fungal PLFA 18:3ω3,6,9, which was only detected at this site in the tree row soil, contributing on average 6.0 mol%. Zhou et al. (2022) assessed 18:1ω9c and 18:3ω3,6,9 as unspecific, so that only 18:2ω6,9 remained as fungal indicator PLFA. This is not justified as none of the three fungal indicator PLFA exclusively occurs in fungi (Joergensen, 2022). To reduce this type of error, the estimation of ergosterol is recommended in the presence of plants.

4.3. Agroforestry effects on AMF

Except at Wendhausen, a marked 12 to 19 % AMF decline was observed in the tree row in comparison with the alleyway, although poplar and willow trees live in symbiosis not only with ectomycorrhizal fungi but also with AMF (Gherghel et al., 2014; Fortin Faubert et al., 2022). In addition, diverse herbaceous layers grow under the tree rows (Beule et al., 2020) with many herb and grass species holding AMF symbiosis (Battie-Laclau et al., 2020). The reason for this decline in the tree rows is unclear, as the omission of tillage increases AMF spores and hyphae abundance (Kabir, 2005; Säle et al., 2015). The current mean contribution is close to the mean of 4.1 mol% reported by Joergensen and Wichern (2008) for 16:1ω5 in arable and grassland soils. The lack of standard organisms might also be the reason for information on AMF not being given (Beule et al., 2020), despite the importance of this microbial group for providing nutrients to the vegetation. The difficulties in finding basic information on one of the oldest fungal phyla might be reason why AMF are often not considered as fungi in PLFA analysis (Bach et al., 2010; Hu et al., 2014; Yang et al., 2017).

AMF are certainly obligate biotrophic as they are unable to excrete extracellular decomposing enzymes. However, they can take up organic components from the soil solution to survive (Hodge, 2014; Hodge et al., 2014), often called cheating (Allison et al., 2014; Joergensen and Wichern, 2018). This ability to survive might be an important reason why AMF PLFA remain in soil for extended periods, even after cell death, e.g., caused by sterilisation (Gryndler et al., 2018; Lekberg et al., 2022). Many organisms that are unable to excrete decomposing enzymes survive in soil by cheating: e.g., AMF, yeasts, algae, and cyanobacteria (Joergensen and Wichern, 2018). The direct experimental evidence for this is small for AMF, but the review of Hodge (2014) and especially the paper of Hodge et al. (2014) gives sufficient evidence for this view.

There is an ongoing discussion on the reliability of PLFA 16:1ω5 as an indicator of AMF (Lekberg et al., 2022; Olsson and Lekberg, 2022), as this AMF has sometimes been detected in substrates where no AMF PLFA should be present in high concentrations, such as cattle faeces (Frostegård et al., 1997). However, also Lekberg et al. (2022) did not present direct evidence that cultured Gram(−) bacteria contain high concentrations of this PLFA 16:1ω5. Zelles (1997) observed no or only traces of PLFA 16:1ω5 in Gram(−) bacteria. A possible but not proven explanation might be the incorrect assignation of a peak as PLFA 16:1ω5 in the chromatogram. In contrast to Frostegård et al. (1997), Meyer et al. (2021) did not find any of this PLFA in cattle faeces. Small shifts in the chromatogram may lead to a wrong assignation, as described by Joergensen and Wichern (2008) for fungal PLFA, suggesting that the

detection of fungal PLFA might be affected by analytical constraints (Elfstrand et al., 2008; Murugan et al., 2021).

In close proximity to the current grassland site Reiffenhausen, Zhou et al. (2022) carried out a ^{13}C pulse-labelling experiment with chambers. In this experiment, the AMF PLFA biomarker 16:1ω5 was specifically enriched with ^{13}C in soil under grassland, i.e., 8 % of total ^{13}C labelled PLFA, which was markedly higher than under willow or oilseed rape. In addition, the AMF PLFA was not related to the PLFA specific for Gram (−) bacteria. However, even if results give unclear impressions, NLFA 16:1ω5 (Lekberg et al., 2022) or even the EL-FAME 16:1ω5 (Acosta-Martínez et al., 2010; Li et al., 2020) can give additional information on AMF, despite their less close relationship with the AMF biomass (Bååth, 2003; Joergensen, 2022). In most cases, the PLFA 16:1ω5 gives sufficient information on AMF (Joergensen and Wichern, 2008; Faust et al., 2017). However, as the majority of PLFA, except those of Actinobacteria, are not fully specific, care should always be taken not to overinterpret PLFA data.

This current uncertainty might be improved by *q*PCR-based approaches (Bainard et al., 2012; Banerjee et al. (2016), combined, e.g., with Illumina amplicon sequencing (Battie-Laclau et al., 2020).

4.4. Agroforestry effects on bacteria

Agroforestry effects on the relative composition of bacterial groups in mol% were smaller than those on fungi. Consequently, the ratio of fungal to bacterial PLFA is significantly higher in the tree row than in the alleyways at 0–5 cm depth. The same is true for the MBC/ergosterol ratio (Beuschel et al., 2019, 2020) as well as the amino sugar based fungal/bacterial necromass ratio at the three poplar sites (Beuschel et al., 2019). In contrast, Beule et al. (2020) did not find any significant differences in the ratio of bacterial to fungal gene copies at the three poplar sites and also the bacterial α-diversity remained largely unaffected by tree rows (Beule and Karlovsky, 2021).

The bacterial PLFA were dominated by Gram(−) bacteria, followed by the Gram(+) phyla Firmicutes and Actinobacteria. Similar contributions of the bacterial groups to total PLFA were presented by Zhou et al. (2022) for the grassland site Reiffenhausen. This contrasts the data of Joergensen and Potthoff (2005), who observed a dominance of Gram (+) bacteria in an arable Cambisol, summarizing the PLFA derived from Firmicutes and Actinobacteria. Both groups were in most cases separately evaluated in soil microbiology over the last decades (Zhou et al., 2022), due to their different response to environmental conditions. Firmicutes respond rapidly to fresh and easily available substrates, whereas Actinobacteria usually show a slow response, but decomposing a broad spectrum of recalcitrant organic components.

The Gram(−) bacteria/Firmicutes ratio showed a small but significant decline at Dornburg and Forst with increasing distance from the tree row, although the differences in environmental conditions in the alleyway transects are less variable than those between the tree row and the alleyways. The reasons for this observation cannot be explained by the current dataset. Some bacteria might respond sensitively to changes in the AMF and BAM composition, as many bacteria belong to fungal helper community (Nasslahsen et al., 2022).

The current PLFA data are not in agreement with the 16S RNA gene copy numbers presented by Beule et al. (2020), who observed that roughly 30 % of the total bacteria gene copy numbers were contributed by Actinobacteria. Firmicutes contributed only an extremely low number of approximately 3×10^8 or 0.001 ‰ to the total bacterial gene copy numbers. Their *q*PCR data were roughly in line with bacterial amplicon sequence variants, which were assigned to 10 dominant bacterial phyla (Beule and Karlovsky, 2021), i.e., 39 % Actinobacteria, 22 % Proteobacteria, 11 % Acidobacteria, 9 % Chloroflexi, and 6 % Planctomycetes. The differences in molecular and PFLA data might be largely caused by differences in gene copy numbers obtained from the genomes of bacteria and archaea (Stoddard et al., 2015) as well as fungi (Baldrian et al., 2013; Heidrich and Beule, 2022). For this reason, the comparison of relative and absolute abundances showed strong discrepancies, indicating that amplicon sequencing alone cannot adequately assess population size and dynamics (Beule et al., 2021). A similar strong mismatch between 16S rDNA metabarcoding and PLFA data, especially for Gram (−) bacteria, has already been observed by Lewe et al. (2021).

Currently, it remains uncertain whether Gram(−) or Gram(+) bacteria dominate the bacterial biomass in soil, although this knowledge is required for the reliable conversion of bacterial muramic acid to bacterial necromass C (Appuhn and Joergensen, 2006; Engelking et al., 2007). It would be helpful to establish appropriate conversion values from bacterial PLFA to bacterial biomass by analyzing cultured bacteria, especially considering that Gram(−) bacteria contain more PLFA in their biomass due to their bi-layered cell membrane.

5. Conclusions

Tree rows shifted the PLFA composition to more ecto-mycorrhizal and saprotrophic fungal biomarkers (18:1ω9, 18:2ω6,9, and 18:3ω3,6,9) of the BAM group (Basidiomycota, Ascomycota and Mucoromycota) in comparison with the alleyways. The bacterial PLFA diversity was higher in the arable alleyways than in the tree rows. The contribution of Gram(−) bacterial PLFA was increased in the tree rows and decreased within the alleyways, whereas that of Firmicutes PLFA decreased. The composition of bacterial PLFA contrasts gene copy numbers obtained by quantitative real time polymerase reaction, which is especially true for the abundance of Actinobacteria. This indicates the need for further investigation of this discrepancy. The differences between arable alley cropping systems with poplar and the grassland alley cropping systems with willow were small, except for the strongest contribution of the BAM group to total PLFA in the soil under willow trees. Agroforestry generally supports the C sequestration potential of mycorrhizal fungi.

Authors contributions

KG, RGJ, MD, and CW contributed to the conception and design of the study. KG and CB conducted the laboratory work. KG, RGJ, HPP, and CW performed the statistical analysis. KG wrote the first draft of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version of the manuscript.

CRediT authorship contribution statement

Katharina Giray: Data curation, Methodology, Writing – review & editing, Formal analysis, Visualization. **Callum Banfield:** Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing. **Hans-Peter Piepho:** Formal analysis, Methodology, Software, Validation, Writing – review & editing. **Rainer Georg Joergensen:** Formal analysis, Funding acquisition, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Michaela Dippold:** Methodology, Supervision, Writing – review & editing. **Christine Wachendorf:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Katharina Giray reports financial support was provided by Bundesministerium für Bildung und Forschung, Germany.

Data availability

Data will be made available on request.

Acknowledgements

The technical assistance of Gabriele Dormann and Karin Schmidt is highly appreciated. This study was supported by the German Federal Ministry of Education and Research (BMBF) in the framework of the BonaRes-SIGNAL project. We also would like to thank Mick Locke for carefully correcting our English.

References

Acosta-Martínez, V., Dowd, S.E., Bell, C.W., Lascano, R., Booker, J.D., Zobeck, T.M., Upchurch, D.R., 2010. Microbial community composition as affected by dryland cropping systems and tillage in a semiarid sandy soil. Diversity 2, 910–931.

Agnihotri, E., Abhishek Bharti, A., Ramesh, A., Prakash, A., Sharma, M.P., 2021. Glomalin related protein and C16:1ω5 PLFA associated with AM fungi as potential signatures for assessing the soil C sequestration under contrasting soil management practices. Eur. J. Soil Biol. 103, 103286.

Allison, S.D., Lu, L., Kent, A.G., Martiny, A.C., 2014. Extracellular enzyme production and cheating in *Pseudomonas fluorescens* depend on diffusion rates. Front. Microbiol. 5, 00169.

Appuhn, A., Joergensen, R.G., 2006. Microbial colonisation of roots as a function of plant species. Soil Biol. Biochem. 38, 1040–1051.

Bååth, E., 2003. The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. Microb. Ecol. 45, 373–383.

Bach, E.M., Baer, S.G., Meyer, C.K., Six, J., 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. Soil Biol. Biochem. 42, 2182–2191.

Bainard, L.D., Koch, A.M., Gordon, A.M., Klironomos, J.N., 2012. Temporal and compositional differences of arbuscular mycorrhizal fungal communities in conventional monocropping and tree-based intercropping systems. Soil Biol. Biochem. 45, 172–180.

Baldrian, P., Větrovský, T., Cajthaml, T., Dobišášová, P., Petránková, M., Šnajdr, J., Eichlerová, I., 2013. Estimation of fungal biomass in forest litter and soil. Fungal Ecol. 6, 1–11.

Banerjee, S., Baah-Acheamfour, M., Carlyle, C.N., Bissett, A., Richardson, A.E., Siddique, T., Bork, E.W., Chang, S.X., 2016. Determinants of bacterial communities in Canadian agroforestry systems: co-occurrence patterns of soil bacterial communities. Environ. Microbiol. 18, 1805–1816.

Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y., van Wezel, G.P., 2016. Taxonomy, physiology, and natural products of *Actinobacteria*. Microbiol. Molec. Biol. Rev. 80, 1–43.

Battie-Laclau, P., Taschen, E., Plassard, C., Dezette, D., Abadie, J., Arnal, D., Benezech, P., Duthoit, M., Pablo, A.-L., Jourdan, C., Laclau, J.-P., Bertrand, I., Taudiere, A., Hinsinger, P., 2020. Role of trees and herbaceous vegetation beneath trees in maintaining arbuscular mycorrhizal communities in temperate alley cropping systems. Plant and Soil 453, 153–171.

Becklin, K.M., Pallo, M.L., Galen, C., 2012. Willows indirectly reduce arbuscular mycorrhizal fungal colonization in understorey communities. J. Ecol. 100, 343–351.

Beule, L., Karlovsky, P., 2021. Tree rows in temperate agroforestry croplands alter the composition of soil bacterial communities. PloS One 16, e0246919.

Beule, L., Lehtsaar, E., Corre, M.D., Schmidt, M., Veldkamp, E., Karlovsky, P., 2020. Poplar rows in temperate agroforestry croplands promote bacteria, fungi, and denitrification genes in soils. Front. Microbiol. 10, 3108.

Beule, L., Arndt, M., Karlovsky, P., 2021. Relative abundances of microbial taxa as determined by amplicon sequencing can be misleading: soil fungal communities in temperate agroforestry as an example. Microorganisms 9, 589.

Beuschel, R., Piepho, H.-P., Joergensen, R.G., Wachendorf, C., 2019. Spatial distribution of soil quality indicators in three poplar-based silvo-arable alley cropping agroforestry systems in Germany. Biol. Fertil. Soils 55, 1–14.

Beuschel, R., Piepho, H.-P., Joergensen, R.G., Wachendorf, C., 2020. Similar responses of soil quality indices to tree implementation and grassland management in a temperate willow-based grassland alley cropping system. Appl. Soil Ecol. 147, 103373.

Bicharanloo, B., Shirvan, M.B., Cavagnaro, T.R., Keitel, C., Dijkstra, F.A., 2022. Nitrogen addition and defoliation alter belowground carbon allocation with consequences for plant nitrogen uptake and soil organic carbon decomposition. Sci. Total Environ. 846, 157430.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Dippold, M.A., Kuzyakov, Y., 2016. Direct incorporation of fatty acids into microbial phospholipids in soils: position-specific labeling tells the story. Geochim. Cosmochim. Acta 174, 211–221.

Djajakirana, G., Joergensen, R.G., Meyer, B., 1996. Ergosterol and microbial biomass relationship in soil. Biol. Fertil. Soils 22, 299–304.

Elfstrand, S., Lagerlöf, J., Hedlund, K., Mårtensson, A., 2008. Carbon routes from decomposing plant residues and living roots into soil food webs assessed with ^{13}C labelling. Soil Biol. Biochem. 40, 2530–2539.

Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biol. Biochem. 39, 2111–2118.

Faust, S., Heinze, S., Ngosong, C., Sradnick, A., Oltmanns, M., Raupp, J., Geisseler, D., Joergensen, R.G., 2017. Effect of biodynamic soil amendments on microbial communities in comparison with inorganic fertilization. Appl. Soil Ecol. 114, 82–89.

Fortin Faubert, M., Labrecque, M., Hijri, M., 2022. Ectomycorrhizal fungi dominated the root and rhizosphere microbial communities of two willow cultivars grown for six-years in a mixed-contaminated environment. J. Fungi 8, 145.

Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14, 151–163.

Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25, 723–730.

Frostegård, Å., Petersen, S.O., Bååth, E., Nielsen, T.H., 1997. Dynamics of a microbial community associated with manure hot spots as revealed by phospholipid fatty acid analyses. Appl. Environ. Microbiol. 63, 2224–2231.

Gherghel, F., Behringer, D., Haubrich, S., Schlauß, M., Fey-Wagner, C., Rexer, K.-H., Janßen, A., Kost, G., 2014. Former land use and host genotype influence the mycorrhizal colonization of poplar roots. Forests 5, 2980–2995.

Golicz, K., Piepho, H.-P., Minarsch, E.-M.L., Niether, W., Große-Stoltenberg, A., Oldeland, J., Breuer, L., Gattinger, A., Jacobs, S., 2023. Highlighting the potential of multilevel statistical models for analysis of individual agroforestry systems. Agrofor. Syst. 97, 1481–1489.

Gryndler, M., Šmilauer, P., Püschel, D., Bukovská, P., Hršelová, H., Hujslová, M., Gryndlerová, H., Konvalinková, T., Jansa, J., 2018. Appropriate nonmycorrhizal controls in arbuscular mycorrhiza research: a microbiome perspective. Mycorrhiza 28, 435–450.

Gunina, A., Dippold, M.A., Glaser, B., Kuzyakov, Y., 2014. Fate of low molecular weight organic substances in an arable soil: from microbial uptake to utilisation and stabilisation. Soil Biol. Biochem. 77, 304–313.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J. 9, 1177–1194.

Heidrich, V., Beule, L., 2022. Are short-read amplicons suitable for the prediction of microbiome functional potential? A critical perspective. iMeta 1, e38.

van der Heijden, M.G.A., Martin, F.M., Selosse, M.-A., Sanders, I.R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol. 205, 1406–1423.

Hodge, A., 2014. Interactions between arbuscular mycorrhizal fungi and organic material substrates. Adv. Appl. Microbiol. 89, 47–99.

Hodge, A., Campbell, C.D., Fitter, A.H., 2014. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413, 297–298.

Hu, Y., Xiang, D., Veresoglou, S.D., Chen, F., Chen, Y., Hao, Z., Zhang, X., Chen, B., 2014. Soil organic carbon and soil structure are driving microbial abundance and community composition across the arid and semi-arid grasslands in northern China. Soil Biol. Biochem. 77, 51–57.

IUSS Working Group WRB, 2022. World reference base for soil resources. International soil classification system for naming soils and creating legends for soil maps. In: International Union of Soil Sciences (IUSS), 4th ed. Vienna, Austria.

Joergensen, R.G., 2022. Phospholipid fatty acids in soil – drawbacks and future prospects. Biol. Fertil. Soil 58, 1–6.

Joergensen, R.G., Emmerling, C., 2006. Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils. J. Plant Nutr. Soil Sci. 169, 295–309.

Joergensen, R.G., Potthoff, M., 2005. Microbial reaction in activity, biomass, and community structure after long-term continuous mixing of a grassland soil. Soil Biol. Biochem. 37, 1249–1258.

Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol. Biochem. 40, 2977–2991.

Joergensen, R.G., Wichern, F., 2018. Alive and kicking: why dormant soil microorganisms matter. Soil Biol. Biochem. 116, 419–430.

Kabir, Z., 2005. Tillage or no-tillage: impact on mycorrhizae. Can. J. Plant Sci. 85, 23–29.

Lacombe, S., Bradley, R.L., Hamel, C., Beaulieu, C., 2009. Do tree-based intercropping systems increase the diversity and stability of soil microbial communities? Agric. Ecosyst. Environ. 131 (1–2), 25–31.

Lawson, G., Dupraz, C., Watté, J., 2019. Chapter 9: Can Silvoarable Systems Maintain Yield, Resilience, and Diversity in the Face of Changing Environments? Reconciling Contemporary Agriculture and Environmental Quality. Academic Press, Elsevier, London, Agroecosystem Diversity, pp. 145–169.

Lekberg, Y., Bååth, E., Frostegård, Å. Edith, Hammer, E., Hedlund, K., Jansa, J., Christina Kaiser, C., Ramsey, P.W., Řezanka, T., Rousk, J., Wallander, H., Monika Welc, M., Olsson, P.A., 2022. Fatty acid 16:1ω5 as a proxy for arbuscular mycorrhizal fungal biomass: current challenges and ways forward. Biol. Fertil. Soils 58, 835–842.

Lewe, N., Hermans, S., Lear, G., Kelly, L.T., Thomson-Laing, G., Weisbrod, B., Wood, S.A., Keyzers, R.A., Deslippe, J.R., 2021. Phospholipid fatty acid (PLFA) analysis as a tool to estimate absolute abundances from compositional 16S rRNA bacterial metabarcoding data. J. Microbiol. Methods 188, 106271.

Li, C., Cano, A., Acosta-Martinez, V., Veum, K.S., Moore-Kucera, J., 2020. A comparison between fatty acid methyl ester profiling methods (PLFA and EL-FAME) as soil health indicators. Soil Sci. Soc. Am. J. 84, 1153–1169.

Meyer, S., Thiel, V., Joergensen, R.G., Sundrum, A., 2019. Relationships between feeding and microbial faeces indices in dairy cows at different milk yield levels. PloS One 14, e0221266.

Meyer, S., Grüning, M.M., Beule, L., Karlovsky, P., Joergensen, R.G., Sundrum, A., 2021. Soil N_2O flux and nitrification and denitrification gene responses to feed-induced differences in the composition of dairy cow faeces. Biol. Fertil. Soils 57, 767–779.

Mungai, N.W., Motavalli, P.P., Kremer, R.J., 2006. Soil organic carbon and nitrogen fractions in temperate alley cropping systems. Comm. Soil Sci. Plant Anal. 37, 977–992.

Murugan, R., Bhople, P., Djukic, I., Zehetner, F., Keiblinger, K., Zimmermann, M., Zechmeister-Boltenstern, S., Joergensen, R.G., 2021. Temperature sensitivity of CO_2

efflux in soils from two alpine elevation levels with distinct bedrock types. Appl. Soil Ecol. 162, 103875.
Nasslahsen, B., Prin, Y., Ferhout, H., Smouni, A., Duponnois, R., 2022. Mycorrhizae helper bacteria for managing the mycorrhizal soil infectivity. Front. Soil Sci. 2, 979246.
Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiol. Ecol. 29, 303–310.
Olsson, P.A., Lekberg, Y., 2022. A critical review of the use of lipid signature molecules for the quantification of arbuscular mycorrhiza fungi. Soil Biol. Biochem. 166, 108574.
Olsson, P.A., Bååth, E., Jakobsen, I., Söderström, B., 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycol. Res. 99, 623–629.
Olsson, P.A., Larsson, L., Bago, B., Wallander, H., van Aarle, I.M., 2003. Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. New Phytol. 159, 1–10.
Piepho, H.P., Edmondson, R.N., 2018. A tutorial on the statistical analysis of factorial experiments with qualitative and quantitative treatment factor levels. J. Agro. Crop Sci. 204, 429–455.
Powlson, D.S., Brookes, P.C., Christensen, B.T., 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biol. Biochem. 19, 159–164.
Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., Van Der Heijden, M.G.A., Oehl, F., 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biol. Biochem. 84, 38–52.
Stenberg, B., Johansson, M., Pell, M., Sjödahl-Svensson, K., Stenström, J., Torstensson, L., 1998. Microbial biomass and activities in soil as affected by frozen and cold storage. Soil Biol. Biochem. 30, 393–402.
Stoddard, S.F., Smith, B.J., Hein, R., Roller, B.R.K., Schmidt, T.M., 2015. rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. Nucleic Acids Res. 43, D593–D598.
Sun, H., Koal, P., Gerl, G., Schroll, R., Gattinger, A., Joergensen, R.G., Munch, J.C., 2018. Microbial communities and residues in robinia- and poplar-based alley cropping systems under organic and integrated management. Agrofor. Syst. 92, 35–46.
Udawatta, R.P., Rankoth, L.M., Jose, S., 2019. Agroforestry and biodiversity. Sustainability 11, 2879.
Unger, I.M., Goyne, K.W., Kremer, R.J., Kennedy, A.C., 2013. Microbial community diversity in agroforestry and grass vegetative filter strips. Agroforestry Systems 87, 395–402.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.
Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction - an automated procedure. Soil Biol. Biochem. 22, 1167–1169.
Yang, S., Xu, Z., Wang, R., Zhang, Y., Yao, F., Zhang, Y., Turco, R.F., Jiang, Y., Zou, H., Li, H., 2017. Variations in soil microbial community composition and enzymatic activities in response to increased N deposition and precipitation in inner Mongolian grassland. Appl. Soil Ecol. 119, 275–285.
Zak, J., Willig, M., Moorhead, D., Wildman, H., 1994. Functional diversity of microbial communities. A quantitative approach. Soil Biol. Biochem. 26, 1101–1108.
Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35, 275–294.
Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fertil. Soils 29, 111–129.
Zhou, J., Huadong Zang, H., Loeppmann, S., Gube, M., Kuzyakov, Y., Pausch, P., 2020. Arbuscular mycorrhiza enhances rhizodeposition and reduces the rhizosphere priming effect on the decomposition of soil organic matter. Soil Biol. Biochem. 140, 107641.
Zhou, J., Li, Z., Shi, L., Kuzyakov, Y., Pausch, J., 2022. Microbial utilization of photosynthesized carbon depends on land-use. Geoderma 428, 116160.