Spatial heterogeneity of soil quality within a Mediterranean alley cropping agroforestry system: Comparison with a monocropping system

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Highlights

- Temperate alley cropping systems induce spatial heterogeneity in soil quality
- Soil quality is improved until 2 m beyond the tree row in the interrow
- 21 years of agroforestry increased the soil quality by 20% compared with monocrop

Abstract

Alley cropping agroforestry systems are complex agroecosystems highlighted for their positive effects on soil quality. However, the potential spatial heterogeneity of soil quality created by tree rows at the plot scale has seldom been studied. The aim of this study was to evaluate soil quality at the plot scale, under tree rows and along transects perpendicular to the tree row and to compare alley cropping systems with monocropping systems. This study was performed on an alley cropping system that combined hybrid walnut trees (21 years old) and peas. Topsoil was sampled at tree rows between 1 and 2, 2 and 4 and 4 and 6.5 m from the tree row in the alley cropping system, as well as in a neighbouring monocropping plot. Physical, chemical and microbiological indicators of soil quality were measured. Tree row implantation induced spatial heterogeneity in the chemical indicators, microbial biomass, activities and community structure at the alley cropping plot scale. Alley cropping not only improved microbiological soil quality indicators within the tree rows but also in the interrows when compared to a monocropping system. These indicators were then integrated into one soil quality index (SQI) built through a statistical approach. The soil quality index was calculated for the monocropping plot and for each position within the alley cropping plot. After 21 years of agroforestry practice, tree rows and permanent grass cover improved the SQI until 2 m in the interrow. Weighted SQIs were calculated relative to the surface area of each location for the entire alley cropping plot (i.e., tree row + interrow positions) and for the entire alley cropping interrow (i.e., removing the tree row surface area). The weighted SQI of the entire alley cropping plot significantly increased compared with that of the monocropping plot.

Keywords: Alley cropping system, spatial heterogeneity, soil quality index, microbial activities, soil organic carbon

1. Introduction

Soil quality can be defined as "the continued capacity of the soil to function within ecological and land-use boundaries, to sustain productivity, to promote the quality of air and water, and to maintain plant, animal and human health" [1]. Soil quality indicators are parameters that are sensitive enough to be modified by land use or management practices [2], [3] and should provide key information concerning the composition, structure and function of the soil [4]. In addition, a combination of physical, chemical and biological indicators must be used to correctly interpret soil quality [5], [6].

Physical indicators are related to the "inherent quality", which is influenced by soil age and past climates [7], while most chemical and all biological indicators can be associated with the "dynamic quality", which is highly sensitive to land use and soil management [8]. Inherent and dynamic soil quality indicators refer to changes over long and medium to short terms, respectively [9]. Among physical soil quality indicators, water storage, bulk density, aggregate stability and texture are the most frequently measured [2]. Chemical indicators can be evaluated to characterize soil fertility and nutrient availability (e.g., soil organic carbon, total N, Olsen P, CEC, pH, exchangeable ions, and N mineralization rate) [10]. Biological indicators of soil quality are necessary to connect abiotic soil properties to soil functioning [2], [11]. Soil microbial biomass, abundance, diversity and activity play key roles as the main drivers of soil organic matter decomposition and nutrient cycling [12], [13]. Soil microorganisms can be studied as soil quality indicators through their biomass (e.g., microbial biomass C), activities (e.g., enzyme activities and substrate-induced respiration) and community structure (e.g., PLFA and sequencing) [14]. A minimum dataset of indicators has to be selected either through expert opinion [15] or through statistical approaches [5], [16] to assess soil quality, avoiding collinearity between indicators. Both methods allow the construction of a soil quality index (SQI), which accounts for a more or less comprehensive set of indicators [10], [17]. The use of aggregated SQIs is currently strongly developed and can improve the comparisons of land use or management effects.

Land management is recognized as one of the main drivers affecting soil quality improvement or degradation [18]. Temperate agroforestry systems comprising a combination of trees and crops are examples of agroecosystems that provide a sustainable alternative to conventional cropping systems, in which plant diversity is low; hereafter, these conventional cropping systems are called monocropping systems. A recent review of European agroforestry practices confirmed their positive effect on ecosystem services; however, this effect was context-dependent [19]. One of the most important ecosystem services promoted by agroforestry systems is their potential capacity for carbon (C) storage, both temporarily in tree biomass through photosynthesis [20] and in the long term in soil organic matter [21]. High inputs of above- and belowground litter in the tree row in alley cropping can provide higher amounts of C and nutrient resources and thereby modify soil microbial communities [22].

Even though a global positive effect of agroforestry on soil quality has been recognized [23], it should be noted that only a small proportion of studies have considered the spatial heterogeneity induced by tree rows. As highlighted by Cardinael et al. [24], future studies should integrate more spatial dynamics and investigate lateral spatial heterogeneities instead of concentrating on only the tree row. The few publications that have considered the potential spatial heterogeneity of soil quality have not allowed us to claim that agroforestry systems significantly improve soil quality at the plot scale or within the interrow. For instance, [25] did not detect differences between tree rows and interrows in microbial biomass C (MBC) and basal respiration or [26] in bacterial diversity or [27] in enzyme activities.

Mungai et al. [28] found significantly more enzyme activities in tree rows than in interrows but no difference within cultivated interrows. The spatial extent to which tree rows and their understorey vegetation strips affect soil quality should be investigated.

In the present work, we evaluated the effect of an alley cropping agroforestry system on the physical, chemical and biological components of soil quality compared to a neighbouring, monocropping system managed in the same way as the interrows in the alley cropping system. We first hypothesized that spatial gradients of soil quality occur between the tree row and middle of the interrow, with the greatest values of soil quality indicators on and close to the tree row and decreasing values with increasing distance from the tree row. Second, we hypothesized that the weighted soil quality of alley cropping, integrating its spatial heterogeneity throughout the plot, is higher than that of the neighbouring monocropping plot.

2. Materials and methods

2.1. Site description

The experimental site of Restinclières is located at Prades-le-Lez, 15 km north of Montpellier, southern France (43°42′15 N, 3°51′41 E). This location has a subhumid Mediterranean climate, with an average temperature of 15.4 °C and average annual rainfall of 658 mm between 2000 and 2016 (Fréjorgues weather station). The soil is a deep Fluvisol (WRB, 2007) with a silty-clay texture. In February 1995, hybrid walnut trees (*Juglans regia* × *nigra* cv. NG23) were planted 4 m apart along east-west rows, with an interrow distance of 13 m. The initial planting density was 208 trees ha^{-1}, but the plot was thinned in 2004 down to a present density of 110 trees ha^{-1}. Adult walnut trees have not been pruned since 2014. The tree rows (2 m wide) were covered with spontaneous herbaceous vegetation, as they have not been ploughed or treated with herbicides since 1995. Each interrow was ploughed to a 20 cm depth every year before the winter crop was sown and was fertilized at an approximate rate of 150 kg N ha^{-1} yr^{-1} (as NH_4NO_3). Most of the time since 2000, durum wheat has been the major crop in rotation, but pea has been introduced every 4 years since 2010, and barley has been introduced since 2015 [29]. Over the 2014-2016 period, durum wheat (*Triticum turgidum durum* cv. Claudio)-barley (*Hordeum vulgare* cv. Augusta)-pea (*Pisum sativum* cv. Igloo) rotation was practised on this 4.6-ha alley cropping plot and on the adjacent 1.4-ha monocropping plot, which has been managed in the same way as the cultivated interrows in the alley cropping since the experimental site was established. All crops were sown between the end of October and the beginning of December and harvested by the end of June/early July.

2.2. Soil sampling

Soil sampling was conducted in April 2016, when pea was growing in the monocropping plot and in the interrows of the alley cropping plot. At this stage, the walnut trees had no leaves yet. In the 1.4 ha monocropping plot, five sampling areas of 1 m^2 each, at least 15 m apart, were identified for soil sampling. In each 1 m^2 surface area, one composite soil sample, based on 4 soil cores (8 cm in diameter) from each angle of the area, was collected from the 0-15 cm layer of topsoil using a root auger. For the alley cropping plot, five transects at least 40 m apart were sampled from the tree row to the middle of the interrow on both the north and south sides of the tree row. Each north-south transect corresponded to 7 positions (35 soil samples in total) (**Fig. A.1**). Soils were sampled in the tree row, with the centre of the 2-m^2 sampling area located on the axis of the tree row, 1 m from the nearest tree. Other sampling areas were located in the interrow between 1 and 2 m from the middle of the tree row, between 2 and 4 m and between 4 and 6.5 m. Soil samples were collected from the 0-15 cm layer of topsoil using a root auger at each angle of a centred subsurface (representing half of the total surface area of the location) (**Fig. A.1**). Areas sampled on the north side are indicated by "N", and those on the south side are indicated by "S". Once collected, the fresh soil samples were passed through a 2-mm sieve, and subsamples were stored at 4 °C for microbial biomass and MicroResp® analyses, which were performed within two weeks after sampling and at -20 °C prior to freeze-drying for PLFA analyses.

2.3. Physical and chemical analyses

Physical and chemical indicators were measured on the north side of the trees (corresponding to 4 positions per transect) and in the monocropping system, i.e., for a total of 25 samples. The moisture content was determined for all samples after drying for 48 h at 105 °C. Bulk density was determined by dividing the soil dry mass by the volume of the root auger. Additional analyses were performed by the national routine soil testing lab of INRAE at Arras (LAS, France). Soil texture was determined after $CaCO_3$ dissolution, and pH was determined in a water extract. The cation exchange capacity (CEC) was determined using the cobalt hexamine chloride method and analysed using inductively coupled plasma-atomic emission spectrometry (ICP-AES) [30]. Soil organic C (SOC) and total N were determined by dry combustion. The available P content was determined colorimetrically after extraction using the Olsen method [31]. Roots were collected from each soil core (15 cm height × 8 cm diameter) and pooled into one composite sample (4 cores) for each area. They were separated into three size classes as follows: 0-2 mm (fine roots), 2-5 mm (medium roots) and > 5 mm (large roots).

2.3.1. Density and particle size fractionation

Density fractionation was performed on the north side of the trees and in the monocropping system, i.e., for a total of 25 soil samples. The fractionation procedure was derived from Roscoe et al. [32]. Briefly, 25 g of soil was shaken with 100 ml of a 1.6 Mg m^{-3} dense sodium polytungstate solution (NaPT) and then centrifuged for 30 min at 6800 *g*. The suspended material was termed the free light fraction (f-LF < 1.6 Mg m^{-3}) and was separated from the supernatant by filtration using a 0.7 µm glass fibre filter. The material recovered on the filter paper was washed with distilled water to remove residual NaPT. New NaPT (density 1.6 Mg m^{-3}) was added to the remaining material. The new suspension was subjected to ultrasonic dispersion (25 J ml) for 3 min. Afterwards, the suspension was centrifuged at 6800 *g* for 30 min, and the supernatant was filtered to recover the occluded light fraction (o-LF < 1.6 Mg m^{-3}) (procedure see above). The remaining material was then separated into two particle-size fractions by wet sieving: 50-2000 µm (sand) and < 50 µm (silt and clay). Before C and N measurements, each fraction was decarbonated by acid fumigation using 12 M HCl in a desiccator for 6 h [33]. After removing calcium carbonate, C and N contents in the total soil, the f-LF o-LF and 50-2000 µm and < 50 µm fractions were analysed by dry combustion with an elemental analyser (Elemental Analyser Vario Pyro Cube). This density fractionation procedure allowed the separation of free soil organic matter (f-LF), soil organic matter associated with minerals (o-LF and 50-2000 µm) and physically protected soil organic matter (< 50 µm).

2.3.2. Non-cellulosic neutral sugar determination

Rhamnose, fucose, mannose, galactose, glucose, ribose, arabinose and xylose were quantified in the total soil, f-LF, o-LF and < 50 µm fractions following the protocol of Rumpel and Dignac [34] modified through the addition of 0.9 ml of 2 M EDTA after hydrolysis with trifluoroacetic acid (TFA) [35]. Briefly, 500 mg for total soil, between 60 and 70 mg for f-LF and o-LF and 600 mg for the < 50 µm size fraction were added to 10 ml of TFA and hydrolysed at 105 °C for 4 h. After hydrolysis, myoinositol was added as an internal standard. Samples were filtered through a glass fibre filter (0.7 μm), and TFA was eliminated by evaporation before derivatization. Aldoses were reduced to their corresponding alditols after the addition of 1 ml of $NaBH_4$ dissolved in dimethyl sulfoxide (DMSO). Acetylation was performed by adding 2 ml of acetic anhydride and 2 ml of glacial acetic acid using methylimidazole (2 ml) as the catalyst. The reaction was stopped after 10 min by adding 7 ml ice-cold deionized water. The derivatised sugar monomers were extracted with 1 ml of dichloromethane. The analyses were performed using a gas chromatograph (HP 6890 GC-FID) equipped with an SGE BPX-70 column (60 m × 0.32 mm internal diameter, 0.25 μm film thickness). The gas chromatography oven temperature program was 200 °C to 250 °C at 8 °C min^{-1} and isothermal at 250 °C for 15 min with helium as the carrier gas.

The sum of extracted monosaccharides from plant-derived hemicellulose and microbial products [36] is hereafter termed "sugars". The proportion of microorganism-derived sugars in relation to plant-derived sugars can be estimated from the ratio of hexose:pentose sugars: (galactose + mannose):(arabinose + xylose), hereafter called GM:AX [37]. GM:AX ratios < 0.5 and > 2 are representative of carbohydrates predominantly derived from plants and microorganisms, respectively [37].

2.4. Soil microbiological indicators

The study plot had an east-west tree row orientation, and we wanted to test whether this orientation could modify soil microbiological parameters, which are sensitive to changes in climatic conditions at the local scale. Accordingly, we measured microbiological parameters on both the north and south sides of the tree row., i.e., for a total of 40 samples.

Microbial biomass C (MBC), N (MBN) and P (MBP) contents were quantified using the chloroform fumigation-extraction technique [38]. Briefly, 10 g (for MBC and MBN) or 2 g (for MBP) of equivalent dry soil was exposed to chloroform vapor for 24 h and then extracted with 40 ml of 0.025 M K_2SO_4 (for MBC and MBN) or with 40 ml 0.5 M $NaHCO_3$ (for MBP), shaken for 45 min, centrifuged (10 min at 2683 *g*) and then filtered through 0.22-µm PTFE filters. Soil organic C and N concentrations in the extracts were measured by a TOC/TON analyser (OI-Analytical, Aurora 1030, College Station, USA). Inorganic P concentrations in the extracts were quantified colorimetrically using the malachite green method [39]. Microbial biomass C, N, and P (MBC, MBN and MBP) contents were calculated from the difference between the chloroform-fumigated and non-fumigated samples. We applied a conversion factor of 0.45 for MBC [40], 0.54 for MBN [41] and a conversion factor of 0.4 for MBP [40]. Data are expressed in mg C, N or P kg $soil^{-1}$. The total C of nonfumigated samples was used to represent dissolved organic carbon (DOC).

Basal and substrate-induced respiration was measured using the MicroResp™ approach [42]. The soil water holding capacity (WHC) was determined using Richard's membrane press at pF 2.5 [43]. Sieved soils were adjusted to 40% of their WHC and were preincubated for 7 days at a temperature of 23 °C ± 2 °C in the dark following the protocol of Bérard et al. [44]. We tested three different soluble C substrates: glucose, trehalose and alanine [44]. In a 96-deep-well microplate, 350 mg of soil sample was distributed, and then 25 µl of each substrate was dispensed in each of the wells at 10 µg substrate mg^{-1} soil. The 96-deep-well microplate was then sealed with a CO_2-trap gel and incubated for 6 h in dark conditions at 23 ± 2 °C. The optical density of each well of the CO_2-trap gel was measured on a fluorometric microplate reader (Victor 3, Perkin Elmer) at 570 nm.

The microbial community structure was assessed using the phospholipid fatty acid (PLFA) method described by [45]. All analyses of PLFAs were performed by Microbial iD, Inc. (Newark, USA). Briefly, lipids were extracted from 5 g of freeze-dried soil by using a modified Bligh-Dyer extraction with 19 ml of extractant. Lipids were separated on a solid-phase extraction column, and phospholipids were eluted with 5 ml of methanol. After evaporation, phospholipids were transesterified to fatty acid methyl esters, extracted in 4 ml of hexan, evaporated again and then analysed using a gas chromatograph (Agilent 6890 Technologies, Wilmington, USA) [45]. PLFA peaks were identified using the MIDI PLFAD1 calibration mix and naming table (MIDI, Inc., Newark, USA). Individual PLFA markers were used to determine the number of peaks identified per position (tree row, 1-2 m $_{N/S}$, 2-4 m $_{N/S}$, 4-6.5 m $_{N/S}$ for alley cropping and monocropping). Gram-positive (GP) bacteria were identified by 13:0 iso, 14:0 iso, 15:1 iso w6c, 15:1 anteiso w9c, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:1 iso w9c, 17:1 anteiso w9c, 17:1 anteiso w7c, 17:0 iso, 17:0 anteiso, 18:0 iso, and 20:0 iso; gram-negative (GN) bacteria were identified by 13:f1 w5c, 13:1 w4c, 14:1 w5c, 15:1 w6c, 16:1 w9c, 16:1 w7c, 17:1 w8c, 17:0 cyclo w7c, 18:1 w7c, 18:1 w5c, 19:1 w8c, 19:0 cyclo w7c, 20:1 w9c, 20:1 w6c, 21:1 w8c, 21:1 w3c and 22:1 w3c (reference table from MIDI, Inc., Newark, DE). The peaks associated with 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 23:0 and 24:0 were considered general indicators [46], and they were not integrated in analyses and were classified as "non-identified peaks" in **Table A.1**. The GP:GN ratio was calculated based on the previously described peaks. A relative measure of the fungi/bacteria (F:B) ratio was calculated by dividing the fungal PLFA marker: 18:2 w6c, 18:1 w9c [46], [47], [48] and 16:1 w5c, the last one representing the arbuscular mycorrhizal fungi (AMF) by the sum of GN and GP bacteria. Actinobacteria were identified by 16:0 10-methyl, 17:1 w7c 10-methyl, 17:0 10-methyl, 18:1 w7c 10-methyl, 18:0 10-methyl and 19:1 w7c 10-methyl. Other unknown peaks were associated with the "others" category and were considered only in the calculation of the relative abundance of each PLFA group. All PLFA markers are listed in **Table A.1**.

2.5. Calculation of the soil quality index (SQI)

We followed the method proposed by Obriot et al. [10] to build an aggregated index of soil quality. This method is based on 4 steps and starts with a dataset from different soil variables and leads to a unitless single score. The first step (step 1, [10]) consisted of the inventory of soil parameters. The second step (step 2, [10]) aimed to produce a minimum dataset (MDS) for each category. Only those parameters that significantly discriminated the various locations within the agroforestry plot (mixed model analysis, $P < 0.05$) were considered relevant [10]. Then, a Pearson correlation analysis was performed to identify correlated parameters (> 0.8) and to avoid redundant information in the aggregated index. For the third step (step 3, [10]), each indicator was normalized between 0 and 1 using the "more is better", "less is better" or "optimum" response curves. For each parameter, the type of response curve was determined according to the literature [10], [49], [50]. For all parameters, we used the "more is better" response curves except for bulk density and Olsen P, for which the "optimum" response curve was chosen [51], [52]. Selected indicators from the MDS were then computed in a principal component analysis (PCA), and the contribution of each indicator to the dimensions of the PCA was calculated. Finally, the SQI was calculated according to Equation 1 and Equation 2 (step 4, [10]), with a weighting of the transformed variables using the PCA eigenvectors and the percentages of total variability explained by each principal component:

<u>Equation 1</u>: $W_i = \sum_{j=1}^{p} \lambda j \times fj$

<u>Equation 2</u>: $SQI = \sum_{i=1}^{n} Si \times Wi$

where fj = relative percentage of total variability attributed to each principal component, λj = sum of squared coordinates on each eigenvector, Si = normalized indicator scores and Wi =

weighted factors. One SQI was calculated for each position within the alley cropping plot, and one for the entire alley cropping (hereafter called weighted alley cropping), weighting each surface area on the north side for the SQI. Another SQI was calculated for the alley cropping interrow excluding the tree row area in the weighted calculation (hereafter called weighted interrow). The last SQI was calculated for the monocropping system.

2.6. Statistical analyses

Mixed models with distance to the tree row as a fixed effect and transect number as a random effect were created for all soil parameters. Normal distribution of residuals and homogeneity of variance were tested by using the Shapiro and Bartlett tests. When necessary, data were Box-Cox transformed (MASS R package). If significant, a Tukey post hoc test was used for pairwise multiple comparisons ($P < 0.05$). Differences between monocropping and each position from the spatial gradient were tested by a one sample t-test ($P < 0.05$) considering the monocropping system as a reference.

A Pearson correlation matrix was calculated to assess relationships between environmental parameters and the abundance of soil microbial functional groups. Redundancy analysis (RDA) was performed to assess the relationship between the soil microbial community composition, i.e., relative abundance of different microbial groups and environmental parameters. Most discriminating variables were selected using a forward procedure, and significance was also tested (100,000 permutations) (vegan R package). All statistical analyses were performed with R software v.3.2.3 (R development Core Team, 2015).

3. Results

3.1. Physical and chemical soil quality

The soil was calcareous with more than 50% calcium carbonate, which explained the alkaline pH (**Table 1**). Levels of Olsen P were systematically low regardless of the position in the alley cropping or monocropping plots. The tree row showed significantly higher values than the interrow positions for the following variables: soil moisture, SOC, DOC, N_{tot}, mineral N, Olsen P, CEC and exchangeable cations.

The largest C content was systematically found in the smallest fraction, i.e., < 50 µm fraction, contributing to more than 50% of the total soil C (**Table 2**). The highest C content was found in the tree rows regardless of the fraction, e.g., within the 50-2000 µm fraction, 3.6 g C kg^{-1} soil was measured in the tree rows versus 2.0 - 2.3 g C kg^{-1} soil within the interrows and 1.5 g C kg^{-1} soil in the monocropping plots. The C:N ratios of f-LF and o-LF were significantly lower under the tree row (18.7 and 16.5, respectively) than under the middle of the interrow (22.8 and 18.7, respectively) (**Table A.2**).

The highest sugar-C concentration was also found in the < 50 µm fraction (1.87 - 2.36 mg sugar C g^{-1} soil) and did not significantly differ among positions in the alley cropping plot or compared with the monocropping plot (**Table 3**). The sugar-C concentration in f-LF under the tree row was significantly (two-fold) higher than that in the middle of the interrow or in the monocropping plot. In the same fraction, the proportion of microorganism-derived sugars in relation to plant-derived sugars (GM:AX ratio) decreased from 0.85 in the tree row down to 0.67 in 4-6.5 m_N and was significantly higher than that in the monocropping plot (0.62).

In the tree row, the total root biomass was more than 2-fold higher than anywhere within the interrow or compared with the monocropping plot (**Fig. A.2**). Significant differences were noticeable for fine- and medium-class roots.

3.2. Biological soil quality indicators

We observed a soil MBC close to 2-fold higher in the tree row (427 mg C kg^{-1}) than in the middle of the interrow (219 mg C kg^{-1} soil) and in the monocropping plot (199 mg C kg^{-1} soil) (**Fig. 1**). The same difference was observed for MBN and MBP. Compared with monocropping, we systematically found a significant increase in MBC, MBN and MBP in the tree row and in 1-2 $m_{N/S}$.

The MB-C:N ratio significantly increased with increasing distance from the tree row, from 6.0 in the tree row to 7.4 in 4-6.5 m_N (**Table A.3**). The MB-C:P ratio was significantly lower under the tree row (24.5) than in 4-6.5 m_N (41.4). Values of MB-C:N and values of MB-C:P were similar in the monocropping plot and close to those in the tree rows.

Basal respiration was significantly increased only in the 1-2 m_S position compared with all others (**Table 4**). Compared with monocropping, glucose-induced respiration was significantly enhanced at all positions in the alley cropping plot except in 4-6.5 m_N. Alanine- and trehalose-induced respiration was significantly higher in the tree row and close to it in the alley cropping plot than in the monocropping plot. Alanine-induced respiration was more than 2-fold higher under the tree row than in the middle of the interrow. The metabolic quotient (qCO_2) was similar across all positions (P > 0.05, **Table 4**).

The PLFA diversity, i.e., the number of detected peaks, decreased with increasing distance from the tree row (**Fig. A.3)**. Actinobacteria abundance was significantly highest in the tree row (2.58 µg PLFAs g^{-1} soil) and similar for the interrow positions and for the monocropping plot (**Fig. 2a**). For GP and GN bacteria, AMF and other fungi, soils under the tree row and in 1-2 m_S showed significantly higher abundance than those in the middle of the interrow or in the monocropping plot (**Fig. 2a, b**). The F:B ratio did not show significant differences between positions within the alley cropping plot or compared with the monocropping plot: all values were approximately 0.30 ± 0.1 (data not shown). The GP:GN ratio was significantly lower in and close to the tree row compared with the interrow or monocropping plot (**Fig. 2a**).

3.3 Soil quality index

Eight indicators, i.e., bulk density, SOC, N_{tot}, CEC, Olsen P, basal respiration, and glucose- and alanine-induced respiration ultimately contributed to the construction of the SQI (**Fig. 3**).

We observed a gradual decrease in the SQI with increasing distance from the tree row (0.76 to 0.53). The monocropping plot had an SQI similar (0.52) to that found in the middle of the interrow. The contribution of each individual indicator to SQI construction was approximately 12%, except Olsen P, which contributed less than 3%. Individual contributions varied according to the position in the alley cropping plot following a same spatial pattern that was similar to the SQI. The weighted SQI of the entire alley cropping plot (0.64) was significantly 20% higher than that of the monocropping plot (0.52), while an increase of 10% of the weighted SQI for the alley cropping interrow (0.57) was not significantly different from that of the monocropping system ($P = 0.08$) (**Fig. 3**).

3.4. Relationships between environmental parameters and microbial community structure

To understand the link between soil environmental parameters and soil microbial community structure, we i) calculated a Pearson correlation matrix between different soil characteristics and diversity, and total PLFA biomass and abundance of each microbial group (**Table A.4**) and ii) performed RDA to identify the determinants of the relative abundance of PLFA markers (**Fig. 4**).

The two first axes explained 45 and 10% of the total variation, respectively (**Fig. 4**). The first axis clearly distinguished the relative abundance of actinobacteria and other GP bacteria, which were positively correlated with silt content, and the relative abundance of AMF, GN bacteria and fungi. The AMF and GN bacteria were positively correlated with sugar content and GM:AX ratio in the f-FL fraction, and fungi were positively correlated with sugar content in the o-LF fraction and negatively correlated with clay content.

4. Discussion

4.1. Soil quality index in agroforestry and monocropping plots

In the present work, the selected indicators for the minimum dataset were in line with expert opinion [2], [53], [54]. Bulk density and SOC content represent important weighted indicators in soil quality assessment for their role in water infiltration, storage and supply, as well as CEC, N and P contents for nutrient storage and supply [11]. Among the selected biological indicators, basal respiration is considered a physiological trait of microbial communities, and glucose-induced respiration is assumed to be proportional to active microbial biomass [44]. In this study, we focused on the microbial component of soil functioning, although we are aware that other biological indicators, such as those related to meso- or macro-fauna, could have been helpful for soil quality assessment [9], [55].

Our aim was to aggregate information obtained using different parameters into one integrative soil quality index based on several soil functions: water, C and nutrient storage and cycling and sustainability of biological activities. In the present work, we initially had a large number of indicators (30 in total), and several methods for SQI calculation offered to us. Comparing three different SQI constructions, i.e., additive SQI, weighted additive SQI and statistically modelled SQI, [56] showed that the last one was more efficient in time and cost.

The method for index calculation proposed by [10] allowed us to objectively select the most relevant indicators to assess changes in physical, chemical and biological soil quality in the studied field plots. Without this statistical approach, we could have overestimated the positive effect of tree rows on soil quality because of the co-variability of the measured indicators. However, this method considers as relevant for the first step of minimum dataset selection only parameters that significantly changed statistically according to the treatments, which could be considered a subjective choice.

This is the first time, to the our knowledge, that soil quality was assessed in temperate alley cropping through the use of an integrative and weighted SQI. We showed a strong positive effect from the 21-year-old trees and accompanying permanent grass cover in the tree rows of the studied alley cropping system on soil physicochemical indicators compared with the interrow and monocropping systems; this effect was restricted to the tree rows (**Table 1**). The increase in SOC content in the tree rows led to an increase in those soil properties that are usually related to it, such as CEC and exchangeable cation contents [10] (**Table 1**).

We showed that the SQI was significantly improved in an alley cropping system in comparison with a monocropping system, considering the spatial heterogeneity induced by these systems. This indicates here that the positive effect from the trees and herbaceous cover on the weighted entire alley cropping SQI was significant beyond the tree rows. The tree rows, which represent approximately 13% of the field plot surface area, yielded a significant beneficial effect on SQI over approximately 20% compared with monocropping (**Fig. 3**). These findings should be put in perspective, as the results depend on one site and one sampling date. Additional sub-annual sampling would be necessary to reject a possible seasonal effect. Even though not significant (P = 0.08), there was a 10% increase in the SQI weighted interrow (i.e., 1-2 m + 2-4 m + 4-6.5 m) compared with the neighbouring monocropping plot. Repeating these indicator measurements in time and increasing the number of replicates would allow us to see if this trend could be significant or not.

4.2. What explains the higher SOC content in the alley cropping system?

In our study, the topsoil of tree rows showed SOC contents 20-30% higher than that of the interrow and 50% greater than that of the monocropping plot (**Table 2**), which is in line with former results at the same study site [57]. Within and close to tree rows, belowground litter, as aboveground litter can also be enhanced, as tree roots have been shown to colonize the topsoil at the same agroforestry site until 1.5 m in the interrow [58]. In addition, a substantial portion of roots can originate from (i) herbaceous cover root systems, which can colonize the topsoil close to the tree row, as shown by [59] in younger walnut tree alley cropping, or ii) weed colonization with low dispersal abilities, originating from herbaceous cover [60]. Perennial plants can indeed allocate up to 10-15% more C belowground than crops [61], [62]. The absence of tillage in the tree rows likely favoured some accumulation of both C and N [63].

The novelty of our study is that it provides greater insight into the pools of C found in the topsoil at various locations in the alley cropping system than in the monocropping system. We demonstrate that the additional C found in the topsoil comes from fractions with a higher C:N ratio (> 16) (**Table A.2**), which is more similar to that of plant debris than that of smaller size fractions with a C:N ratio < 10, i.e., close to the C:N ratio of soil microbial communities and soil organic matter [64], [65]. These coarser fractions can be more easily mineralized by microorganisms, suggesting a less stable C pool.

The increased GM:AX ratio in the f-LF fraction from the middle of the interrow to the tree row suggests that a higher proportion of microbial-derived carbohydrates was present in the tree row (**Table 3**). This may have induced higher and faster microbial turnover based on more organic matter recycling through microorganisms and could indicate that the production of organic compounds is easier to stabilize [66], [67]. The RDA confirmed that the increased GM:AX ratio favours more fast-growing microorganisms, i.e., microbial communities with higher GN bacteria proportions (**Fig. 4**).

4.3. Spatial heterogeneity of microbial soil properties in the alley cropping system

Our work demonstrated that an agroforestry system such as the one we studied here may considerably impact soil microbial communities through its biomass, activity and structure, resulting in the creation of within-plot spatial heterogeneity.

Higher microbial biomass and activities in and close to the tree row may represent a higher potential capacity to decompose organic matter and improve nutrient cycling. MicroResp® appeared not to be the most appropriate method to evaluate the activity of microorganisms in the carbonate-rich soil; however, as carbonate content was similar everywhere, it allowed us to compare positions. Our qCO_2 results indicate a similar physiological activity of the microbial biomass regardless of the position within the agroforestry plot [68]. This result was surprising because of the different tillage management practices between the tree row and interrow [69]. However, [70] showed in a meta-analysis that similar qCO_2 values were observed for tilled and no-tilled plots in long-term (10 years) plots. This might indicate that qCO_2 is not sensitive to soil tillage or that microorganisms from the tree row are, at the time of our sampling, as efficient in using C as those in the interrow or in the monocropping system.

In tree rows, the MBC/SOC ratio was significantly higher than that in the interrow or in the monocropping plot (i.e., 23 versus 17, data not shown). This indicates that the MBC increase in tree rows was not merely due to the increase in SOC but also to some properties of the soil organic matter (e.g., greater C availability) within tree rows that favour the development of an abundant soil microbial biomass [71].

Compared with the monocropping system, the microbial biomass and substrate-induced respiration were more significantly higher on the south side of the tree row than on the north side in the alley cropping plot (**Fig. 1, Table 4**). This result underlines the potential legacy effect of microclimate created in this alley cropping agroforestry system. Dufour et al. [72] showed in the same plot that photosynthetically active radiation can be reduced by 38% at 3 m on the north side when the tree canopy is well developed (end of June). Our soil sampling was performed in April before walnut tree budburst began. At this stage, they showed that the reduction in incident light was negligible (10%) compared with that in June (50%). This suggests that the slight difference observed between the north and south sides of the tree rows was due to a legacy effect of the 21-year-old practice.

We found that trees and the herbaceous grass community (dominated by weeds, *Bromus* and *Torilis* species) in the tree rows increased PLFA diversity and biomass. These two parameters were highly correlated with pH (negatively), soil moisture, DOC and total N, as observed by [48] and [22]. In our case, an additional correlation with root biomass highlighted its potential key role in shaping microbial community dynamics (**Table A.4**).

In the present study, we observed that tree rows favour a higher proportion of GN bacteria than GP bacteria, leading to a lower GP:GN ratio. Environments rich in C and available N are known to promote soil GN bacteria [73], which have high affinity for recent plant-derived C compounds [74]. The RDA also suggested a correlation between the sugar content in f-LF and the GN bacterial proportion (**Fig. 4**). In contrast, GP bacteria have been shown to be less affected by low C availability [73]. Actinobacteria and other GP bacteria are known to be able to feed on more complex polymers, such as older organic matter [48]. This could explain their higher proportion in the middle of the interrow and in the monocropping system. Variation in the GP:GN ratio suggests that microorganisms could be more copiotrophic in the tree row than in the interrow of the alley cropping system as the GP:GN ratio increases with decreasing C availability in soil [75] **(Fig. 2a**).

Moreover, high-recalcitrant litter inputs, such as walnut leaves, i.e., litter known to be rich in tannins and lignin [76], could have favoured the fungal pathway within or close to the tree row, while we did not observe this. We hypothesize that the herbaceous cover under the tree row adding labile litter and rhizodeposits buffers the recalcitrant effect of walnut litter. These results raise the question of the importance of considering herbaceous cover in future alley cropping studies.

5. Conclusion

Through the design of our sampling strategy, we evaluated changes in soil quality according to the distance from the tree rows in the interrow of an alley cropping system and compared them with a neighbouring monocropping system. The use of an integrative soil quality index related to water and nutrient storage and to the support of biological activity simplified the comparison of the various positions within the alley cropping plot and with the monocropping plot. After 21 years, soil quality was significantly improved in the tree rows compared with the interrow positions and monocropping. The tree rows and its permanent herbaceous cover enriched the soil in organic matter through an increased input of above- and belowground litters and possibly through enhanced rhizodeposition. The SQI was significantly greater under tree rows and beyond until a 2-m distance from the rows, which was clear evidence of spatial heterogeneity within the cultivated interrows. For the first time, we showed that by considering the SQI of the weighted entire plot of the alley cropping system that soil quality was significantly improved compared with that of the monocropping system.

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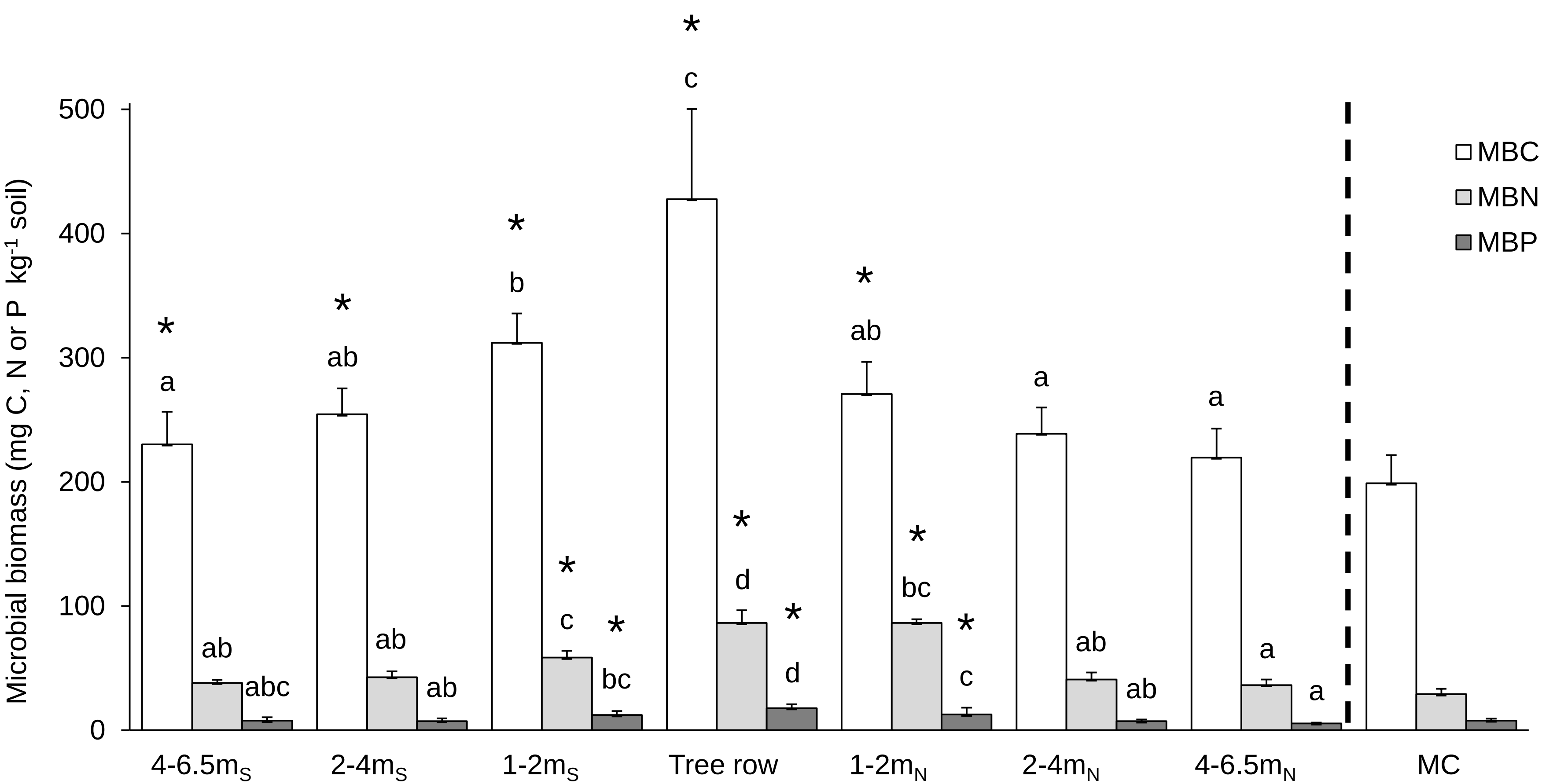
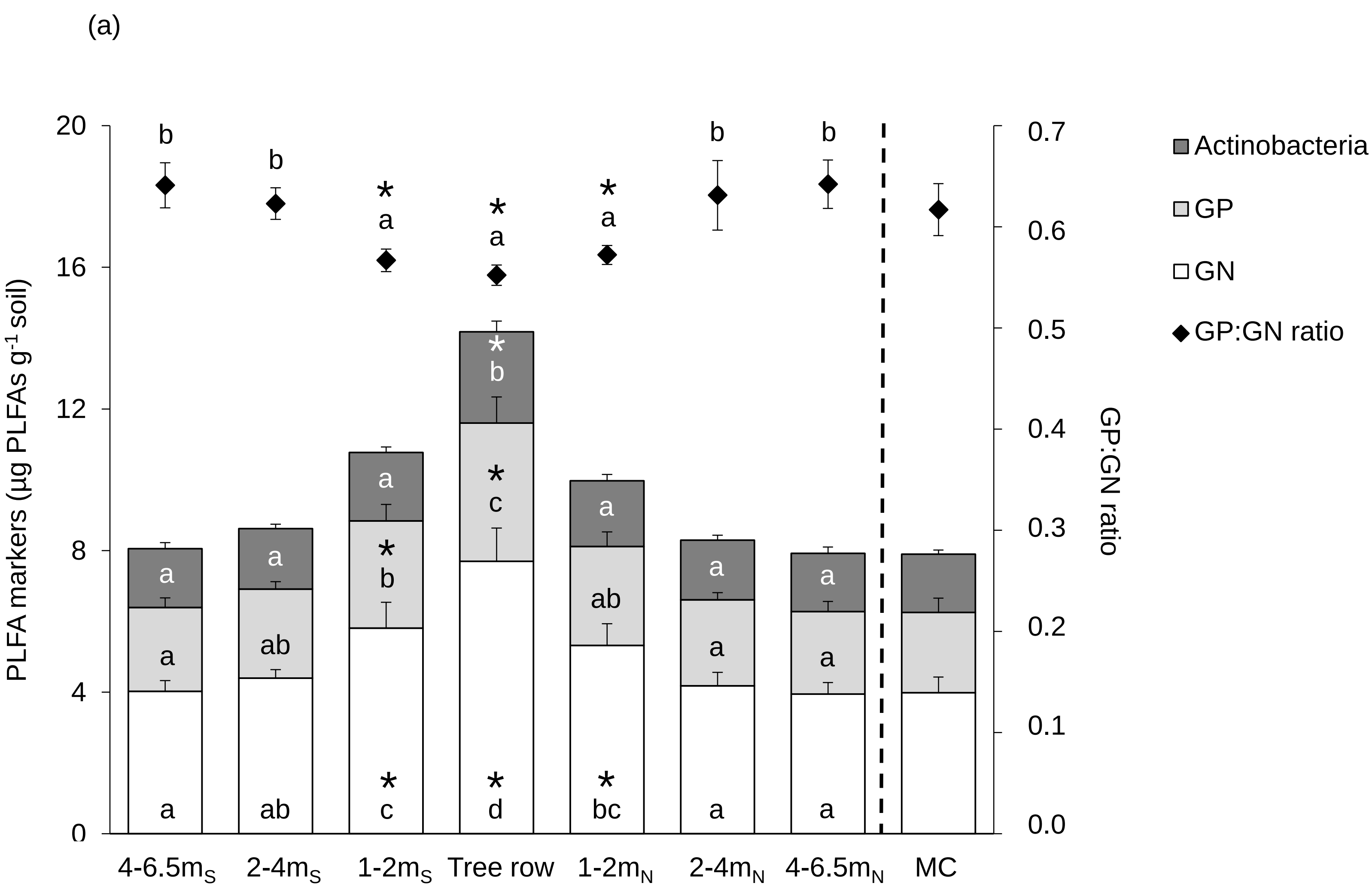
Figure 1



Microbial biomass (mg C, N or P kg^{-1} soil)
500
400
300
200
100
0
MBC
MBN
MBP
*
a
ab
abc
ab
ab
b
c
bc
d
ab
bc
c
a
ab
ab
a
a
4-6.5m$_S$
2-4m$_S$
1-2m$_S$
Tree row
1-2m$_N$
2-4m$_N$
4-6.5m$_N$
MC

Figure 2a



(a)
PLFA markers (µg PLFAs g-1 soil)
GP:GN ratio
20
16
12
8
4
0
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Actinobacteria
GP
GN
GP:GN ratio
4-6.5mS
2-4mS
1-2mS
Tree row
1-2mN
2-4mN
4-6.5mN
MC
b
b
a
a
a
b
b
a
a
a
b
a
a
a
a
a
ab
b
c
ab
a
a
a
ab
c
d
bc
a
a

Figure 2b

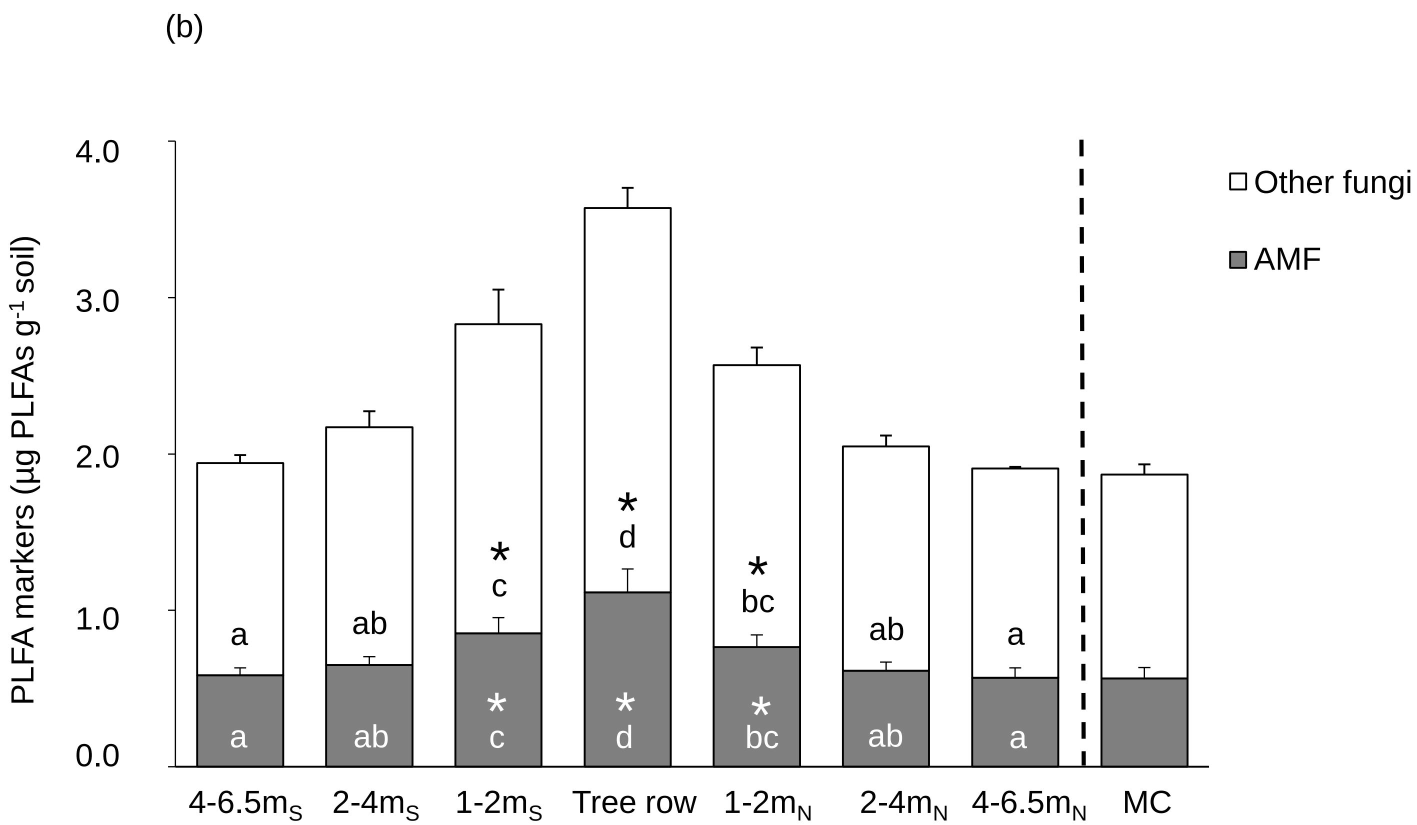
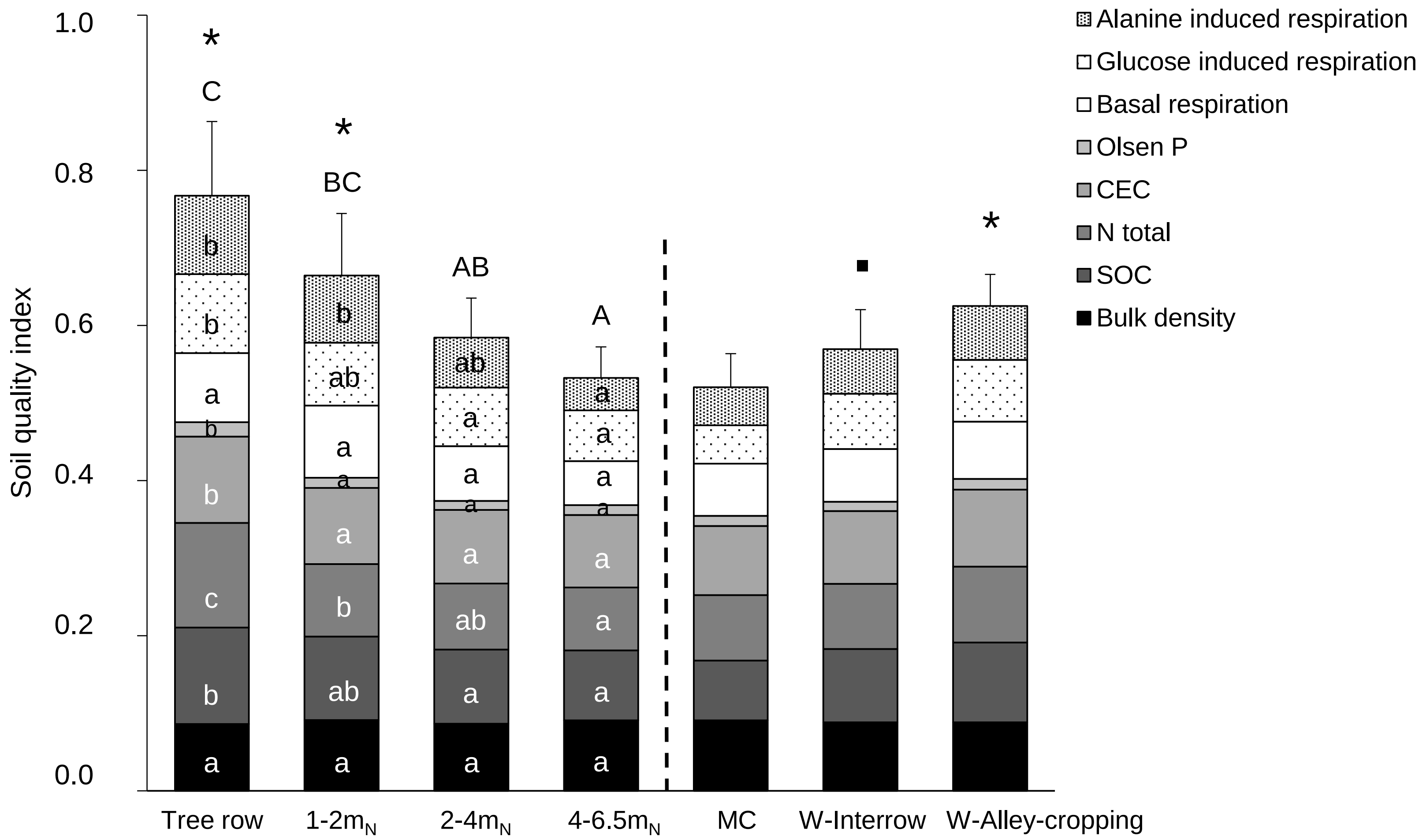

(b)
PLFA markers (µg PLFAs g-1 soil)
4.0
3.0
2.0
1.0
0.0
Other fungi
AMF
a
ab
* c
* d
* bc
ab
a
a
ab
* c
* d
* bc
ab
a
4-6.5mS
2-4mS
1-2mS
Tree row
1-2mN
2-4mN
4-6.5mN
MC

Figure 3



1.0
0.8
0.6
0.4
0.2
0.0
Soil quality index
*
C
*
BC
AB
A
*
Alanine induced respiration
Glucose induced respiration
Basal respiration
Olsen P
CEC
N total
SOC
Bulk density
Tree row
1-2m$_N$
2-4m$_N$
4-6.5m$_N$
MC
W-Interrow
W-Alley-cropping

Figure 4

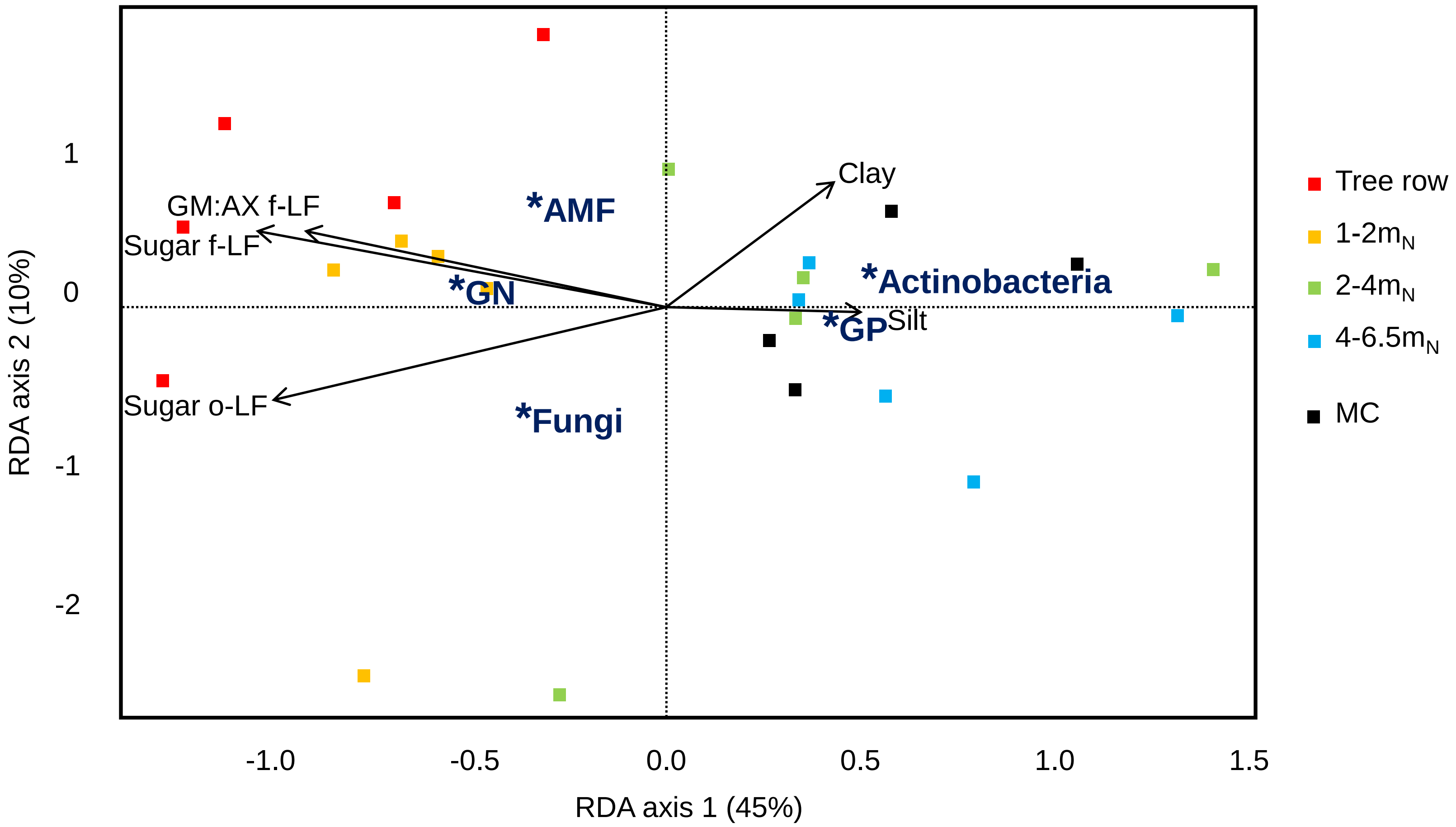

1
0
-1
-2
RDA axis 2 (10%)
-1.0
-0.5
0.0
0.5
1.0
1.5
RDA axis 1 (45%)
Clay
GM:AX f-LF
Sugar f-LF
Sugar o-LF
Silt
*AMF
*GN
*Actinobacteria
*GP
*Fungi
Tree row
1-2m$_N$
2-4m$_N$
4-6.5m$_N$
MC

Table 1

Parameter	Tree row	1-2 m_N	2-4 m_N	4-6.5 m_N	MC
Clay (< 2 µm) (g kg^{-1} soil)	178 ± 9 ^{a}	173 ± 24 ^{a}	186 ± 7 ^{a}	184 ± 13 ^{a}	177 ± 10
Silt (2-50 µm) (g kg^{-1} soil)	157 ± 24 $^{a\,*}$	165 ± 43 $^{a\,*}$	157 ± 23 $^{a\,*}$	158 ± 17 $^{a\,*}$	208 ± 16
Sand (50-2000 µm) (g kg^{-1} soil)	109 ± 18 $^{a\,*}$	101 ± 22 ^{a}	105 ± 25 ^{a}	103 ± 22 ^{a}	76 ± 20
Bulk density (g cm^{-3})	1.24 ± 0.1 $^{a\,*}$	1.41 ± 0.1 ^{a}	1.23 ± 0.1 $^{a\,*}$	1.30 ± 0.1 ^{a}	1.43 ± 0.1
CaCO3 (g kg^{-1} soil)	539 ± 11 ^{a}	545 ± 22 ^{a}	540 ± 26 ^{a}	539 ± 18 ^{a}	527 ± 20
Moisture	14.3 ± 1.8 $^{c\,*}$	13.3 ± 1.1 $^{bc\,\bullet}$	11.8 ± 0.7 ^{a}	11.9 ± 0.9 ^{b}	11.4 ± 0.6
pH_{water}	8.40 ± 0.05 $^{a\,*}$	8.52 ± 0.04 $^{b\,*}$	8.55 ± 0.03 ^{b}	8.57 ± 0.05 ^{b}	8.58 ± 0.03
SOC (g kg^{-1} soil)	19.0 ± 3.5 $^{b\,*}$	16.4 ± 4.5 ^{ab}	14.6 ± 3.0 ^{a}	13.8 ± 2.6 ^{a}	11.8 ± 1.0
DOC (mg kg^{-1} soil)	34.5 ± 5.6 $^{b\,*}$	23.4 ± 2.8 ^{a}	17.4 ± 4.9 ^{a}	18.1 ± 3.2 ^{a}	20.3 ± 2.6
N_{tot} (g kg^{-1} soil)	1.8 ± 0.2 $^{c\,*}$	1.4 ± 0.1 ^{b}	1.3 ± 0.1 ^{ab}	1.2 ± 0.1 ^{a}	1.1 ± 0.1
C:N	10.9 ± 1.6 ^{a}	11.4 ± 2.0 ^{a}	11.2 ± 2.3 ^{a}	11.5 ± 3.9 ^{a}	10.2 ± 3.1
Mineral N (mg kg^{-1} soil)	31 ± 5 $^{b\,*}$	16 ± 4 $^{a\,*}$	11 ± 2 ^{a}	11 ± 2 ^{a}	11 ± 2
Olsen P (mg kg^{-1} soil)	8.3 ± 2.1 ^{b}	5.8 ± 1.2 ^{a}	5.2 ± 1.6 ^{a}	5.6 ± 2.3 ^{a}	5.8 ± 1.3
CEC ($cmol^{+}$ kg^{-1} soil)	14.7 ± 1.9 $^{b\,*}$	13.0 ± 1.6 ^{a}	12.5 ± 1.4 ^{a}	12.4 ± 1.3 ^{a}	11.8 ± 0.4
Exch. Ca ($cmol^{+}$ kg^{-1} soil)	14.7 ± 1.6 $^{b\,*}$	13.3 ± 1.5 ^{a}	13.1 ± 1.1 ^{a}	12.8 ±1.1 ^{a}	12.5 ± 0.5
Exch. Mg ($cmol^{+}$ kg^{-1} soil)	0.73 ± 0.12 $^{b\,*}$	0.47 ± 0.10 $^{a\,*}$	0.39 ± 0.09 $^{a\,*}$	0.38 ± 0.07 $^{a\,*}$	0.29 ± 0.02
Exch. K ($cmol^{+}$ kg^{-1} soil)	0.51 ± 0.08 $^{b\,*}$	0.42 ± 0.08 $^{ab\,*}$	0.36 ± 0.08 ^{a}	0.35 ± 0.07 ^{a}	0.31 ± 0.02

Table 2

Fraction	Tree row	1-2 m_N	2-4 m_N	4-6.5 m_N	MC
f-LF (g C kg^{-1} soil)	3.6 ± 1.3 $^{b\,*}$	2.1 ± 0.9 ^{a}	1.5 ± 0.5 ^{a}	1.3 ± 0.5 ^{a}	1.2 ± 0.3
o-LF (g C kg^{-1} soil)	2.2 ± 2.2 $^{b\,*}$	1.7 ± 1.7 $^{ab\,*}$	1.4 ± 1.4 ^{a}	1.2 ± 1.2 ^{a}	1.0 ± 1.0
50-2000 µm (g C kg^{-1} soil)	3.6 ± 0.9 ^{b}	2.3 ± 0.3 ^{ab}	2.0 ± 0.2 ^{a}	2.0 ± 0.1 ^{a}	1.5 ± 0.1
<50µm (g C kg^{-1} soil)	12.0 ± 2.1 $^{b\,*}$	9.0 ± 1.8 ^{a}	8.6 ± 1.9 ^{a}	7.9 ± 1.8 ^{a}	7.8 ± 1.0

Table 3

Fraction	Tree row	1-2 m_N	2-4 m_N	4-6.5 m_N	MC
Total (mg sugar C g^{-1} soil)	4.4 ± 0.2 [b]	4.1 ± 0.1 [a]	4.0 ± 0.2 [a]	4.0 ± 0.2 [a]	4.0 ± 0.3
f-LF (mg sugar C g^{-1} soil)	0.57 ± 0.19 [b *]	0.35 ± 0.11 [a]	0.32 ± 0.07 [a]	0.28 ± 0.04 [a]	0.29 ± 0.08
o-LF (mg sugar C g^{-1} soil)	0.38 ± 0.05 [a *]	0.31 ± 0.07 [a *]	0.29 ± 0.20 [a]	0.22 ± 0.09 [a]	0.16 ± 0.06
< 50µm (mg sugar C g^{-1} soil)	2.26 ± 0.4 [a]	2.15 ± 0.2 [a]	1.87 ± 0.7 [a]	2.10 ± 0.2 [a]	2.36 ± 0.1
GM:AX Total	0.95 ± 0.06 [a]	0.96 ± 0.04 [a]	1.00 ± 0.04 [a]	0.99 ± 0.04 [a]	0.97 ± 0.05
GM:AX f-LF	0.85 ± 0.15 [b *]	0.73 ± 0.09 [ab]	0.71± 0.09 [ab]	0.67 ± 0.09 [a]	0.62 ± 0.08
GM:AX o-LF	0.95 ± 0.03 [a]	0.91 ± 0.07 [a]	0.97 ± 0.04 [a *]	0.95 ± 0.04 [a]	0.90 ± 0.03
GM:AX < 50µm	1.05 ± 0.01 [a]	1.03 ± 0.02 [a]	1.04 ± 0.02 [a]	1.02 ± 0.02 [a]	1.03 ± 0.01

Table 4

Substrate	Tree row	1-2m$_N$	1-2m$_S$	2-4m$_N$	2-4m$_S$	4-6.5m$_N$	4-6.5m$_S$	MC
Water	0.57 ± 0.09 ^{ab}	0.59 ± 0.20 ^{ab}	0.72 ± 0.25 ^{b}	0.45 ± 0.12 ^{ab}	0.38 ± 0.11 ^{a}	0.36 ± 0.10 ^{a}	0.35 ± 0.06 ^{a}	0.43 ± 0.16
Glucose	2.65 ± 0.47 ^{b*}	2.12 ± 0.50 ^{ab*}	2.49 ± 0.50 ^{b*}	1.97 ± 0.41 ^{ab*}	1.98 ± 0.31 ^{ab*}	1.72 ± 0.41 ^{a}	1.81 ± 0.30 ^{ab*}	1.30 ± 0.21
Trehalose	1.99 ± 0.57 ^{a*}	1.80 ± 0.61 ^{a*}	1.98 ± 0.51 ^{a*}	1.39 ± 0.35 ^{a}	1.44 ± 0.22 ^{a*}	1.17 ± 0.52 ^{a}	1.11 ± 0.14 ^{a}	1.05 ± 0.19
Alanine	1.50 ± 0.56 ^{c*}	1.03 ± 0.61 ^{abc*}	1.44 ± 0.50 ^{bc*}	0.95 ± 0.20 ^{abc}	0.99 ± 0.34 ^{abc}	0.62 ± 0.16 ^{a}	0.72 ± 0.17 ^{ab}	0.73 ± 0.12
qCO_2	0.22 ± 0.02 ^{a}	0.28 ± 0.05 ^{a}	0.29 ± 0.08 ^{a}	0.24 ± 0.09 ^{a}	0.19 ± 0.05 ^{a}	0.21 ± 0.03 ^{a}	0.20 ± 0.03 ^{a}	0.33 ± 0.11