

Soil health in temperate agroforestry: influence of tree species and position in the field

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Abstract

Alley cropping agroforestry - whereby tree rows are integrated in crop plots - is considered as a lever for the agroecological transition. Its benefit for enhancing soil functioning is rarely studied. We studied soil health in a 25 years temperate agroforestry plot cultivated with barley (*Hordeum vulgare*) according to two factors: i. the position to the tree row; and ii. the tree species. Soil health was assessed in three positions (in the tree row; in the crop alley next to the tree row and at 6.5m from the tree row), for three contrasted tree species (*Acer monspessulanum*, *Fraxinus* sp., *Pyrus communis*) using two integrative methods based on soil biological activity (Biofunctool®, MicroResp™). The position factor explained soil health differences the best: mean indexes were found 1.6 times higher in the tree row than in both positions in the crop alley, especially the structure maintenance function was impacted (indexes in the tree row = 0.21 to 0.26; indexes in the crop alley = 0.11 to 0.17). Tree species had less impact on soil health and impacted only carbon dynamics and microbial catabolic profiles. Our study invites to consider spatial organization and tree species to optimize soil ecosystem services in agroforestry systems.

Keywords: Alley cropping; soil health; spatial heterogeneity; Biofunctool®; MicroResp™

Introduction

The agroecological transition of farming systems (Altieri et al. 2015) relies largely on the mobilization of ecological processes to enhance ecosystem services (Gaba et al. 2015; Rey et al. 2015). One pathway for this transition involves increasing biological diversity by planting trees in simplified cropping systems (Jose 2009; Duru et al. 2015; Torralba et al. 2016). Agroforestry, i.e. trees are combined with crops and/or livestock on the same plot (Nair 1985), is one of the main lever to increase biodiversity in farm plots. Currently a wide diversity of agroforestry systems covers about 9% of European agricultural lands with most of these systems located in the Mediterranean regions (den Herder et al. 2017). In France, alley cropping agroforestry systems, where trees are planted in rows between crop alleys, is gaining farmers' attention as tree and herbaceous vegetation growing in the tree rows provide novel semi-natural habitats that enhance biodiversity and spatiotemporal heterogeneity in farm plots (Torquebiau 2000). Such systems are often developed by the integration of tree rows within arable systems, especially with winter crops to avoid light competition between trees and crops in the summer (Dufour et al. 2013).

When trees and the underlying herbaceous vegetation are introduced, a combination of direct (litterfall, presence of perennial herbaceous cover, associated fauna) and indirect (introduction of shade, reflectance and microclimate changes) effects impact the function of the agroecosystem and provide ecosystem services and disservices (Quinkenstein et al. 2009; Dollinger and Jose 2018). Studies

conducted in temperate environments revealed a beneficial effect of alley cropping systems on carbon sequestration and soil fertility, thereby optimizing resource use (Tsonkova et al. 2012). Results regarding other services such as crop production are more heterogeneous, including even negative effects when trees and crops are competing for resources such as light (Artru et al. 2017; Pardon et al. 2018). In alley cropping systems, the soil under tree rows is untilled and mostly covered with spontaneous perennial vegetation. Tree rows are thus relatively undisturbed compared to crop alleys. Alley-cropping agroforestry systems differentially disturb and therefore increase the spatial and functional heterogeneity of soils. This has been made particularly evident in recent studies focusing on the evolution of separate soil functions according to the distance to tree rows - such as carbon sequestration (Cardinael et al. 2015), physicochemical properties, and various biotic assemblages (Beuschel et al. 2019; Boinot et al. 2019; Battie-Laclau et al. 2020; D'Hervilly et al. 2020). By combining physical, chemical, and microbiological indicators of soil quality, Guillot et al. (2021) recently found increased soil quality in alley cropping plots compared to monocropping ones. In addition, planted tree species could also impact the soil given their functional traits. The crown architecture and leaf phenological traits have an impact on the abiotic environment, including temperature, humidity, and intercepted radiation under the canopy (Cardinael et al. 2018). Studies have also highlighted the impact of litterfall quantity and quality as a driver of biotic functioning in the soil ecosystem (Faucon et al. 2017; D'Hervilly et al. 2020). Tree species functional traits were recently shown to have effects on the biophysical properties of tropical soils within cocoa agroforests (Sauvadet et al. 2020), but few studies have explored these links in temperate agroforestry systems (Faucon et al. 2017). Soil health concept is currently widely discussed in the literature (Baveye, 2021; Janzen et al., 2021; Lehmann et al., 2020; Powlson, 2021). A healthy soil is here defined according to Kibblewhite et al. (2008) : « a healthy soil is one that is capable of supporting the production of food and fiber, to a level and with a quality sufficient to meet human requirements, together with continued delivery of other ecosystem services that are essential for maintenance of the quality of life for humans and the conservation of biodiversity ». Kibblewhite et al. (2008), Janzen et al. (2021) and Thoumazeau, Bessou, Renevier, Trap et al. (2019) highlight the importance of working on integrative approaches to assess soil health. These methods focus on the results of interactions between organisms and physicochemical properties, rather than on an additive description of biotic and abiotic soil components (e.g. direct biodiversity measurement, quantification of stocks, etc.). It is these complex biotic and abiotic interactions within the soil which determines how it ultimately functions (Kibblewhite et al. 2008; Thoumazeau, Bessou, Renevier, Trap et al. 2019). Soil health assessments must therefore simultaneously incorporate several criteria to take into account the system's complexities, synergies and trade-offs between functions (Brauman and Thoumazeau 2020). Functional assessment methods such as Biofunctool® (Thoumazeau, Bessou, Renevier, Trap et al. 2019) and MicroResp™ (Campbell et al. 2003) may be used to deal with this conceptual framework by focusing on process driven by soil biodiversity rather than on the intrinsic soil properties. Biofunctool®

evaluates the three essential soil functions (i) structure maintenance (ii) carbon transformation and (iii) nutrient cycling; and MicroRespTM enables to analyse the activity of soil's microbial catabolic profiles. The present study investigates soil health heterogeneity in temperate alley cropping agroforestry systems according to two factors: (i) the position relative to the tree row and (ii) the planted tree species. The study - carried out in southern France - was performed in one of the few mature and species-diverse agroforestry systems in Europe (25-year-old trees). This plot is conducted with a typical crop rotation of arable monocrop systems of the French Mediterranean region. Thus, studying impact of position to the trees and tree species on soil health within this plot enables us to elucidate the role of the introduction of trees within a typical arable system of the region. Furthermore, our study newly considers the impact of tree species on soil health of a temperate agroforestry system and compares it to the already documented impact of the distance to the tree row by using two complementary integrative methods of soil health assessment based on soil biological activity. We hypothesize that (i) the soil is healthier as it is near and within the tree row and (ii) tree species contrasts have indirect effects on soil functioning through their influence on local microclimate.

Material and Methods

Site description

The study was carried out in June 2020 at the Domaine de Restinclières agroforestry site (43°42'59.4''N; 3°51'33.0''E), 15 km north of Montpellier in southern France (Figure 1a). Subhumid Mediterranean climatic conditions prevail, with 14.4 °C average temperature and 1078 mm average rainfall recorded between 1999 and 2019¹. The soil is a silty-clay Fluvisol (Cardinael et al. 2015) with a flat topography. The average measured soil texture is 61% clay, 37% silt and 1.2% sand. The soil is calcareous with 515 g kg⁻¹ CaCO₃ and an average pH of 8.8. The study was conducted on an agroforestry plot (Figure 1b) that was established in 1995 to study the behaviour of about 30 tree species. The trees were planted in rows that are oriented East-West, in order to maximize the differences in light received between both sides of the tree row, with 4–6 m spacing between trees and 13 m spacing between rows. The tree rows (2 m wide) had not been tilled since the plantation and were covered with spontaneous vegetation, mainly composed of grasses (*Bromus* sp., *Avena fatua* and *Elymus repens*), that had not been mowed or crushed for several years. The cropping system—typical of arable systems in the region—involved a rotation of pea (*Pisum sativum* cv. Igloo), winter durum wheat (*Triticum turgidum durum* cv. Claudio), and spring or winter barley (*Hordeum vulgare*). Exception was made for 2020 as the barley had been sown after one year of fallow. Until 2018, the soil was ploughed every 3 years to 20 cm depth and a mineral fertiliser was applied at a rate of 150 kg N ha⁻¹ per year (except when pea was cultivated). Due to fallow the previous year, the plot was not ploughed or amended with organic or mineral fertilizers in 2020.

¹ CLIMATE-DATA.ORG for Prades-le-Lez consulted in October 2021 available at <https://fr.climate-data.org/europe/france/languedoc-roussillon/prades-le-lez-65221/>

Choice of tree species for the study

The tree species selection criteria for the study were: (i) contrasting functional traits that may differently affect how the soil functions (Table 1), and (ii) presence in sufficient numbers in the plot to enable replication (see section 2.3). Three species met these criteria: (1) Montpellier maple (*Acer monspessulanum*), (2) ash (*Fraxinus* sp.), including flowering ash (*F. ornus*) and narrow-leaved ash (*F. angustifolia*) with similar functional traits, and (3) wild pear (*Pyrus communis*).

Sampling design

Five pairs of trees per species were selected, with each pair corresponding to two adjacent trees spaced 4 m apart (Figure 1b). For each studied tree pair, soil health measurements were obtained at three positions in relation to the tree row: (i) in the tree row (TR) at equal distance between the two trees, and in the crop alley, (ii) within the crop alley, close to the tree row (C1: 0.5 m from the border of the tree row) and (iii) in the centre of the crop alley (C2: 6.5 m from the border of the tree row) (Figure 1.c). C1 and C2 were located north of the TR to maximize the influence of the tree canopy shade. As previous studies observed few differences in soil biophysical properties between the middle of the crop alley and control monocrop plots (D'Hervilly et al. 2020; Guillot et al. 2021), we considered the point C2 as a control that is only negligibly impacted by trees for our study. Field measurements and soil sampling were carried out in June 2020 at 45 sampling points (3 tree species x 3 positions x 5 replicates). At that time, barley seeds were already developed and maturing. Barley was harvested around a month after field measurements and soil sampling. Exact yields for year 2020 could not be recorded, but a study conducted on a neighboring plot in 2007 and 2008 revealed that yields in alley-cropping agroforestry plots of the Domaine de Restinclières were up to 50% less important in the tree shade compared to a monocrop plot (Dufour et al. 2013).

The tree pairs were not randomly distributed in the experimental plot. Soil textures and their effects on the experimental indicators were measured to assess possible co-effects of inherent soil properties related to this sampling design and showed that changes in soil texture did not explained variations of results on measured indicators. Variations observed in our soil health dataset were therefore mainly explained by the position and tree species factors.

Characterization of environmental states associated with the trees and the positions

Planted trees impact the biotic and abiotic environment in the plot (Quinkenstein et al. 2009; Dollinger and Jose 2018), which could explain the soil heterogeneity in the agroforestry plot. Several environmental variables were recorded at each sampling point to gain insight into the impacts of plant cover biomass, ground shade, soil temperature, soil moisture, soil organic carbon, total soil nitrogen and C/N ratio (0–10 cm soil layer).

Aboveground plant biomass except trees (crop, spontaneous vegetation, litter) was sampled at each point on a 25 x 25 cm surface and weighed after 24 h of oven drying at 60 °C. Tree shade was estimated by a percentage canopy cover score measured using a forest densitometer, as described by Lemon (1956).

Tree shade measurements were performed in late June 2020. At this time, tree canopies are well developed and have maximal impact on the reduction of photosynthetically active radiation (Dufour et al. 2013). Temperature data loggers (iButton[®]) were placed at 2 cm depth to monitor soil temperature over a 6-day period (25 June 2020 to 30 June 2020). For material reasons, this monitoring could only be carried out with three replications per species selected throughout the experimental plot. Soil was collected in each sampling points from the 0–10 cm layer and then oven dried for 48 h at 105 °C to measure soil moisture.

Soil organic carbon (Corg) and total nitrogen (Ntot) measurements were carried out in the laboratory on soil samples from the 0–10 cm layer. These were sieved to 2 mm and air dried, and then ground to 200 µm before elemental analysis (Thermo Fisher Scientific Flash 2000; 0.5 µg sensitivity). Corg in the carbonated soil samples was determined by subtracting the total carbon values from the mineral carbon values obtained using a Bernard calcimeter (NF ISO 10693).

Multicriteria soil health assessment via Biofunctool[®]

The set of nine indicators of the Biofunctool[®] method (Thoumazeau, Bessou, Renevier, Trap et al. 2019) was used at each of the 45 sampling points. The protocols for each method are described in Thoumazeau, Bessou, Renevier, Panklang et al. (2019) and the indicators are listed in Table 2.

Soil structure maintenance was assessed by two indicators describing soil aggregate stability: (i) at the surface (0–2 cm; AggSurf) and (ii) at shallow depths (2–10 cm; AggSoil). After air drying, stability was assessed by immersing the soil aggregates in water and agitating them for specific times according to the procedure described by Herrick et al. (2001). The scores ranged from 0 to 6, where 6 indicates high aggregate stability. The Beerkan test (adapted from Lassabatère et al., 2006) was used to measure soil infiltration rate. Ten 310 mL volumes of water were poured into a 20 cm dia. cylinder placed at 2–3 cm depth. The infiltration rate was then determined by the slope of the linear regression relating the water volume to the time at soil saturation. Finally, soil structure was assessed by assigning a visual score to the different layers of a 25 cm thick soil core (VESS, Guimarães et al. 2011), where the highest score (5) indicates a compact soil.

The nutrient cycling function was evaluated by measuring NO₃⁻ and NH₄⁺ ions (Nmin) extracted from fresh soil. 50 g of fresh soil was placed in a 180 mL KCl (1M) solution and then hand-shaken for 5 min at a rate of one shake per second. Soil **nitrogen (N)** dynamic was approximated by quantifying NO₃-fixation on 6 cm x 2 cm anion exchange membranes placed at 8 cm depth for 13 days (AEMNO3) (Qian and Schoenau 2002; Le Cadre et al. 2018). Membrane elution was carried out by 5 min hand-shaking at a rate of one shake per second in a 35 mL KCl (1M) solution. All solutions were then assayed in a continuous flow analyzer (SAN++, Skalar, Breda, The Netherlands)—the results are in mg N kg⁻¹ soil for the Nmin indicator and in µg N-NO₃⁻ cm⁻² d⁻¹ for the AEMNO3 indicator.

Regarding the soil carbon transformation, soil **carbon (C)** accumulation was estimated using the permanganate oxidizable carbon (POXC) analysis method (Weil et al. 2003), which measures the labile fraction of soil organic carbon resulting from partial oxidation (Culman et al. 2012). Soil organism

activity was assessed using the bait-lamina test (Lamina, von Törne 1990), where a set of eight 16-hole-bearing plastic strips were filled with organic substrate and inserted vertically in the soil at each sampling point (2 rows of 4 strips spaced 20 cm apart). The strips were removed after 13 days and then each hole was scored (0 = no visible substrate degradation; 0.5 = partial degradation; 1 = total degradation). The scores were averaged per sampling point and expressed in % deg d⁻¹. Finally, CO₂ released by soil microorganisms was determined by the SituResp[®] method (Thoumazeau et al. 2017), whereby a colour-based CO₂-sensitive gel was incubated in a 250 mL airtight jar with 100 g of fresh soil for 24 h. Gel colour changes related to CO₂ levels. KMnO₄ solution absorbance for POXC were read using a field spectrophotometer (SpectroVis, Vernier).

Functional microbial profiles assessment via MicroRespTM

Soil catabolic activities, i.e. basal respiration and respiration induced by different carbon substrates, were measured in the laboratory using the MicroRespTM technique (Campbell et al. 2003; Bérard et al. 2012). Soil samples were dispensed into a DeepWellTM 96-well microplate after moisture adjustment to 40% of the soil maximum water holding capacity. 25 µL of water (for basal respiration measurement) or carbon substrate (120 g L⁻¹) was added to each well. A CO₂-capture colorimetric microplate with a perforated silicone seal (for well individualization) was placed opposite each of these microplates. Each system was placed under a press and incubated for 6 h in the dark at 23 °C ± 1 °C. Colorimetric microplate absorbance was then measured at 570 nm. A calibration curve of CO₂ concentration versus absorbance in the system space determined by gas chromatography was obtained by a regression model to calculate CO₂ emission rates. Respiration induced by six carbonaceous substrates (two carbohydrates: cellobiose and trehalose; two amino acids: alanine and glycine; the conjugate base of carboxylic acid: malate; a phenol: catechol) was measured to study the soil sample catabolic profiles.

Statistical analysis

All statistical analyses were performed using R software (R Development Core Team, 2008, R 3.6.1). Linear models were used to analyse the effects of the position and species factors on biotic and abiotic environmental variables and Biofunctool[®] and MicroRespTM indicators. Interactions between the species and position factors were always found to not be significant. Effects on the studied variables were considered independently in the final models.

An ANOVA was performed on each model when the residual normality and homoscedasticity assumptions were verified by a Shapiro-Wilk test and Levene test (car package; Fox and Weisberg 2019). If one of these conditions was not verified, a Kruskal-Wallis test was performed for means comparison. When significant findings were obtained (p < 0.05), a post-hoc test was performed according to the Tukey method after ANOVA, or the Bonferroni method after Kruskal-Wallis tests. For each linear model, contributions of each factor to the overall model were calculated by dividing the sum of squares related to the factor under consideration using the R² coefficient of determination.

After analysing the variables separately, a multivariate analysis was performed using principal component analysis (PCA) (FactoMineR package; Lê et al. 2008).

For Biofunctool®, weights were assigned to each indicator to consider each of the three soil functions equally (Pheap et al. 2019). The coordinates of individuals on dimensions with an eigenvalue > 1 were retained to construct the soil health index according to equation 1 and equation 2 (Obriot et al. 2016; Thoumazeau, Bessou, Renevier, Panklang et al. 2019).

$$Wi = \sum_{j=1}^p \lambda_j \times f_j \text{ (equation 1)}$$

$$SQI = \sum_{i=1}^n Si \times Wi \text{ (equation 2)}$$

Where Wi are weighted factors calculated thanks to the relative percentage of total variability to each principal component of the PCA (fj) and the sum of squared coordinates on each eigenvector (λj) and Si are the normalized indicator scores.

For the normalization of the indicator scores, high scores were assumed to reflect better soil health for each indicator, except for the soil structure indicator (VESS) which had an optimal score of 2.5 (Pheap et al. 2019; Brauman and Thoumazeau 2020). Once the soil health index was calculated, ANOVA followed by Tukey post-hoc tests were performed to analyse variance in the total scores and scores per soil function (structure maintenance, nutrient cycling, carbon transformation). The species factor for each soil function was analysed by separating the scores obtained in the tree rows (TR) from those obtained in the crop alleys (positions C1 and C2). For MicroResp™, a PCA was performed on values obtained for the six substrates tested after normalization by the sum of the respirations induced by each substrate (Bérard et al. 2011).

Results

Effect of the position and the species on the measured environmental characteristics

The position factor had a highly significant effect ($p > 0.001$) on most variables describing the environmental conditions (Table 3). It explained more than 85% of the soil temperature variability (at 06:00 and 15:00), soil moisture, soil C/N, soil organic C and total N. At each TR, C1 and C2 position, the temperature varied cyclically throughout the day. At the coldest time (06:00), the mean temperature in the middle of the crop alley (C2) was 2.5 °C higher than near the trees (TR and C1). At the hottest time (15:00), the mean temperature in the middle of the crop alley (C2) exceeded 30 °C, while it ranged from 22 °C to 28 °C in C1, and was 22.5 °C in TR. Apart from the soil temperature, where a gradient was noted between the three positions, the position effect on the other variables could only be explained by differences between the tree rows (TR) and the crop alleys (C1 and C2). The soil moisture was similar in C1 and C2 (resp. 18.8% and 16.4%) and was nearly 25% lower in TR. Similar differences were found for soil organic C (TR = 3.77%; C1 = 2.31% and C2 = 2.27%) and total N (TR = 0.37%; C1 = 0.25% and C2 = 0.25%).

Differences between tree species regarding the environmental characteristics were less obvious (Table 3). Only shade variations were explained by the species factor (species effect contribution to $R^2 = 82\%$), with denser shade prevailing under ash trees than under maples, while pear trees provided an intermediate amount of shade. Biomass of the tree row understory was the only environmental parameter

that responded similarly to both the position ($R^2 = 45\%$) and species ($R^2 = 55\%$) factors (Table 3). Biomass in the TR - where spontaneous perennial vegetation prevailed - was twofold greater than the biomass noted in the crop alleys (C1 and C2). Moreover, biomass under maple trees was twofold greater than that found under ash and pear trees.

Univariate study of the Biofunctool® indicators

The results obtained for the Biofunctool® indicators and their sensitivities to the two tested factors are presented in Table 4. The soil structure maintenance indicators (AggSurf, AggSoil, Beerkan and VESS) varied according to the position factor only. They had a marked sensitivity to this factor, with a contribution to $R^2 > 89\%$. The observed variation could mainly be explained by differences between the TR and crop alley (C1 and C2) scores. Concerning the nutrient cycling indicators, the Nmin indicator responded to the position factor only, with a contribution to $R^2 = 85\%$. The AEMNO3 indicator showed quite similar results. Finally, only two “carbon transformation” indicators responded to the position factor, with contributions of this factor’s $R^2 > 92\%$: POXC and SituResp®. Lamina was the only indicator to respond strongly and highly significantly to the tree species factor (with a contribution to $R^2 = 92\%$).

Multivariate study of the Biofunctool® indicators

Position effects on the Biofunctool® indicators

Figure 2 shows the results of the first two dimensions from the PCA performed on all Biofunctool® indicators. These two dimensions explained more than 60% of the dataset variability. The first dimension represented 40.1% of the variability and was mainly related to the SituResp, POXC, Beerkan and Nmin variables, which respectively contributed to 19.7%, 18.1%, 14.8% and 14.2% of this dimension (Figure 2a). For this dimension, the SituResp, AggSurf, AggSoil and Beerkan indicators were positively correlated with each other, whereas the VESS indicator was negatively correlated with these variables. This dimension highlighted a separation of the sampling points into two groups: TR points and crop alley sampling points (Figure 2b). TR points were mainly related to significant SituResp, POXC, Beerkan and Nmin indicator values. Figure 3 shows the soil health index results, which ranged from 0.29 to 0.86, with significant differences between the TR and crop alley (C1 and C2) score. In TR, the means ranged from 0.55 to 0.75. In the crop alleys, C1 and C2 positions had similar average results and ranged from 0.35 to 0.43.

Species effects on the Biofunctool® indicators

The second dimension of the PCA accounted for 19.7% of the variability and was mainly explained by the AEMNO3 indicator results (59.8%) (Figure 2). Otherwise, the third dimension represented 13.2% of the dataset variability and was mainly explained by the Nmin (60.2%) and lamina (22.4%) indicator values. As the AEMNO3 and lamina indicators are species-sensitive (Table 4), the second and third dimensions account for the responses to different species.

As the total values of the soil health index was similar for C1 and C2 positions, the species effects on the different soil functions could be assessed separately in the TR and crop alleys (C1 and C2) (Figure

4). For all three soil functions, in particular the structure maintenance and carbon dynamics functions, the mean TR values were higher than in the crop alleys. The soil structure maintenance and nutrient cycling functions results did not differ according to the species factor. The carbon dynamics function was species-sensitive in the crop alleys (Figure 4f). The values recorded under maple trees (mean = 0.13) were lower than those under ash (mean = 0.17) and pear (mean = 0.16) trees. A similar non-significant trend was noted in TR, with soil health indices under pear trees being higher than those under maple trees (Figure 4c).

MicroResp™ study of the soil microbial catabolic profiles

Basal respiration measured with the MicroResp™ technique confirmed the sensitivity to position factor observed with the Biofunctool® results, with values obtained in TR soils (mean: 0.49 $\mu\text{g C-CO}_2 \text{ g}^{-1}\text{soil h}^{-1}$) being significantly higher than in the crop alley soils (means of 0.34 and 0.38 $\mu\text{g C-CO}_2 \text{ g}^{-1}\text{soil h}^{-1}$ at C1 and C2 points, respectively) ($p < 0.05$). Moreover, a significant effect of species on basal respiration was noted in the alley, with higher values obtained near pear trees. A similar trend is observed in the TR but it was not significant.

PCA of catabolic profiles based on respiration induced by the six carbon substrates revealed that 60.0% of the variance could be accounted for by the first two dimensions (Figure 5). Dimension 1 (40.4% of the variance) was mainly explained by the glycine, alanine and malate variables, which contributed respectively to 32.1%, 28.6% and 19.6% of this dimension (Figure 5a). This dimension highlighted the species effects on the catabolic profiles and differentiated the soil samples near pear trees from those near ash and maple trees (Figure 5b). Dimension 2 (19.61% of the variance) was mainly explained by the trehalose variable, which contributed to 48.3% (Figure 5a). This second dimension did not make any distinction between individuals with regard to the species or position factors (Figures 5b and 5c).

Discussion

Position relative to the tree row: the main factor explaining soil health variability

Soil health in the surface layer (0–10 cm) varied mostly with the position in the plot. Major differences in soil health were observed between the tree row and the crop alley area, with a higher soil health level noted within the tree rows. This result is consistent with previously reported findings (D'Hervilly et al. 2021; Guillot et al. 2021), even within younger 5–8 year-old agroforestry systems (Beuschel et al. 2019). All soil functions—especially the soil structure maintenance function—showed sensitivity to the position factor.

The tree rows are undisturbed compartments with permanent vegetation cover. This improved the soil structure, as illustrated in this study by the better infiltration capacity and greater aggregate stability of the soils (Arshad et al. 1999; Pagliai et al. 2004). Conversely, the crop area was ploughed and had a much lower plant biomass level and larger bare soil surface. Thus planting tree rows could help address issues regarding erosion, soil exploration by roots, as well as soil water and nutrient supplies (Stöcker et al. 2020).

Measurements directly related to biological activity, such as permanganate oxidizable carbon (POXC), basal soil respiration (SituResp[®]) and metabolic activity (MicroResp[™]), had higher values in the tree rows (TR) than in the crop alleys. This suggested that soil biological activities were more intense in TR, as also observed by Cardinael et al. (2019) ; D'Hervilly et al. (2021, 2020) and Marsden et al. (2019). These results could be linked to higher organic matter accumulation in TR due to higher input of litter, branches, fine tree roots and vegetation in this compartment (Pardon et al. 2017). Higher levels of available N in the tree row relative to the crop alley might be accounted for by higher organic content and microbial activity as well by a lower N uptake in the tree row. Our findings confirmed the role of trees in helping to engineer ecosystems (Jones et al. 1994): these modify the environment and establish environmental conditions more favourable to biological activity (lower temperature and higher humidity). In our study, the shade data did not differ according to the position relative to the tree row, which was in contradiction with previously published findings (Dupraz et al. 2018). This could be partially explained by the fact that the applied method involved combined monitoring of two tree rows in the centre of the crop alleys. The soil microbial catabolic profiles (MicroResp[™] method) also did not depend on the position. This result is consistent with Guillot et al. (2021) and surprisingly shows that the microclimate induced by the trees as well as differences in soil management between the crop-alley and the tree row did not affect soil microbial catabolic profiles. In the study conditions, the observed effects of TR on carbon transformation seemed thus more quantitative (process intensity) than structural - thereby having little impact on the soil microorganism functional profiles.

In the crop alleys, no difference in the soil health index was noted as a function of the position within the crop alley (C1 vs C2), regardless of the species studied. This was also the case for all of the studied indicators related to microbial activity (MicroResp[™]). This was in agreement with the findings of many previous studies focused on spatial heterogeneity in soil biological and chemical parameters with regard to alley cropping systems in different soil-climate settings and with different species (Beuschel et al. 2019; D'Hervilly et al. 2020; D'Hervilly et al. 2021). Some of these authors explained this environmental compartmentalization by the homogeneous litter distribution noted when the height of the studied trees was greater than the crop alley width (Peichl et al. 2006; Bambrick et al. 2010; Cardinael et al. 2015). In our case, the height of all trees was less than the crop alley width (13 m), thereby implying an irregular litter distribution in the crop alleys. The lack of tree litter in C2 would thus be offset by the higher crop biomass recovery found in the middle of the cropping alleys (Pardon et al. 2018). Another hypothesis is that tillage of the crop alley and crop residue burial would intensify litter mineralization and reduce the long-term effects of litter deposition on the soil surface in C1 (Six et al. 1999; Balesdent et al. 2000). Furthermore, impact of crops and rotation on soil health is non-negligible compared to soil management (Congreves et al., 2015). Crop functional traits of barley such as root depth, branching, turnover and composition might explain part of the soil health indexes obtained in the crop-alley and play a role in the uniform responses of C1 and C2 results. Future research is thus

required to understand the implication of crops, rotations and management practices on soil health in crop alleys of agroforestry systems.

Under similar conditions as our study, Guillot et al. (2021) recently highlighted a gradient of decreasing soil quality with increased distance to the trees. This effect was not observed in our experiment possibly because of the few positions tested in our study. Guillot et al. (2021) observed a soil health gradient in the first 2m starting from a tree, which lies beyond C1 in our experiment. This soil health gradient might also be impacted by the differences in tree species (and relative inputs), the annual state of the crop rotation, and soil texture. It seems important to further understand the conditions under which the soil health in the cropping alley would be affected by trees in alley cropping systems.

Functional diversity of planted tree species: a side effect on carbon transformation

Tree species with different functional characteristics (height, crown shape, leaf area, see Table 1) were selected. While the species selected had a relatively minor impact on most of the soil functions studied, we detected differences with regard to the carbon dynamics function (Biofunctool®), microbial respiration, and catabolic profiles (MicroResp™). These differences were observed in crop alleys (C1-C2) and similar trends are observed in the tree row (TR). The differences could be explained by differences in the quantity and quality of litter generated by the tree species (Mary et al. 2020). Of the three species studied, pear trees had the most positive impact on soil carbon dynamics due to their higher basal respiration. Pear trees affected the activity of the soil microbial community and its functional diversity. The catabolic profiles of soil microbes associated with pear trees featured high respiratory activity linked to amino acids (alanine and glycine) and malate—substrates that may derive from root exudates and fruits. This difference in catabolic profiles could also be linked to the difference in the quantity and quality of litter under the pear trees. Further analysis of leaf C/N and chemical composition could also potentially explain this enhanced carbon dynamic under pear trees.

In the crop alley with maple trees, the soil carbon dynamics function was significantly lower than the results obtained near pear and ash trees, which could mainly be explained by the soil fauna activity (Lamina; partial $R^2 = 0.92$) (Figure 4). Of the species studied, maple differed from the other two species by its shade and higher crop alley temperatures (average crop alley temperatures $> 30^\circ\text{C}$ during the three hottest hours). This thermal stress, which was greater than for the other two species, could have a negative impact on how soil fauna functions, thereby affecting the soil carbon transformation dynamics (Guillot et al. 2019).

In our study, no species sensitivity was observed regarding the nutrient cycling function score. In tropical conditions, Sauvadet et al. (2020) had noted marked species-specific differences in nitrogen cycling. Their study was based on a more contrasted species range than ours, including atmospheric nitrogen-fixing plants. We nevertheless noted an effect on the AEMNO3 indicator. This highlights the limitations of aggregating indicators at the function level in the Biofunctool® scoring approach. The most marked differences in the AEMNO3 indicator were found in TR, with a greater quantity of NO_3^- captured by anion exchange membranes placed under the pear trees. We observed that soil microbial

communities under this species were more adapted to the catabolism of nitrogenous forms (alanine and glycine), which can lead to nitrate generation. This corroborates the previously formulated hypothesis that litter under pear trees would have a higher nutrient supply capacity than other litters.

From soil health assessment to an alley cropping system design tool

This study involved an integrative evaluation of three soil functions, thereby enabling us to assess the impact of two factors— i. position to the trees and ii. tree species—on in soil health variability in alley cropping systems. These results could help to identify options for designing future agroforestry systems. Plot's compartmentation (tree row vs. crop alley) is the factor explaining the most soil health variability in the studied system. A higher soil health was found within the tree row (TR) compared to the crop alley. These results are consistent with previous findings (Beuschel et al. 2019; D'Hervilly et al. 2021; Guillot et al. 2021). Considering that TR takes up around 15% of the surface of the studied plot, alley-cropping agroforestry enabled to enhance soil health on a large surface compared to a monocropping plot. Yet, TR are uncropped areas. Therefore, reducing the distances between TR or widening them to improve soil health in the system would greatly reduce the cropping area in the plot and be counterproductive for the plot's agricultural productivity. Such observation highlights that designing alley-cropping agroforestry needs to consider trade-offs between various ecosystem services delivered to farmers. As soil structure is the function that is the most highly impacted by spatial heterogeneity, farmer's support could be geared towards practices that limit soil structure degradation in crop alleys and so close soil health's gap between the TR and crop alleys. For instance, crop management sequences involving no-till and permanent coverage could be effective to meet this objective (Arshad et al. 1999; Pagliai et al. 2004; Pheap et al. 2019).

Furthermore, soil health within an agroforestry plots seems to be under the effect of tree's induced microclimate. Such climatic conditions impact microbial activity (Guillot et al., 2019). Therefore, considering tree density and TR orientation regarding local climatic conditions seems to be important for enhancing soil health within an agroforestry plot.

The studied agroforestry site was unsuitable for a random sampling design and independent replicates. However, this unique and 25-years-old agroforestry site enabled us to determine the effects of three tree species on soil health. A species effect was noted on the soil C transformation function with Biofunctool®, on nitrate fixed on ion exchange membranes (AEMNO3) as well as on soil microbial catabolic profiles with the MicroResp™ technique. Although we could not test direct effect of litter inputs, we hypothesized that tree species with higher litter inputs and quality enhance C transformation and nutrient cycling functions, which is consistent with Guillot et al. (2019). Our study highlights that tree species have an impact on the soil health of alley cropping agroforestry systems in consistence with other rare studies (Dawud et al. 2017; Sauvadet et al. 2020). It would therefore now be essential to look deeper into the relationships between tree functional traits, associated microclimatic conditions, management of tree rows, biodiversity and soil health. The ultimate goal would be to establish generic links to be able to design systems tailored to specific contexts (Martin and Isaac 2018).

Conclusion

This study revealed that overall soil health was highly heterogeneous throughout temperate agroforestry systems. Soil health varied the most depending on the position relative to the tree row: the soil quality was better in the tree row than in the crop alley. This result highlights the effect of cultivation operations in the overall crop alley. We also observed tree species and associated vegetation effects on the soil carbon dynamics function measured by Biofunctool® and on microbial catabolic profiles measured by MicroResp™. This may be related to the quantity and quality of the organic matter supply and to the microclimatic conditions induced by the characteristics of the trees and their associated spontaneous vegetation in the plot. The findings in this study call for further investigation on the importance of choosing optimal tree species when designing agroforestry systems by taking a look at the links between species and soil health in a wider range of settings.

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Declaration of interests

No potential conflict of interest was reported by the authors.

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Table 1. Characteristics of the studied tree species. Mean ($\pm$ SD). Values in the table were either measured or sourced from the literature. NM = not measured.

Species characteristics	Maple (<i>A.monspessulanum</i>)	Ash (<i>Fraxinus</i> spp.)	Pear (<i>P. communis</i>)	Measurement methods	Source of non-measured data
Height (m)	7.06 ($\pm$ 0.72)	12.77 ($\pm$ 1.20)	7.30 ($\pm$ 0.92)	Measured on 10 individuals (5 pairs) per species (dendrometer)	
Trunk circumference (cm)	65.5 ($\pm$ 9.89)	78.5 ($\pm$ 10.63)	67.1 ($\pm$ 25.40)	Measured on 10 individuals (5 pairs) per species (DBH method at 150 cm height)	
Crown shape	Dome	Tapered	Pyramidal	Pers. obs.	
Root depth (cm)	100 (for <i>A. campestre</i>)	NM	100	-	Kattge et al. 2020 (no data for <i>Fraxinus</i> spp.)
Growth-to-establishment rate (survey: 1996-2001)	Slow	Rapid	Medium	-	Pers. com.
Plant relative growth rate ($\text{g g}^{-1} \text{d}^{-1}$)	0.098 ($\pm$ 0.01) (for <i>A. campestre</i>)	0.090 ($\pm$ 0.04)	0.165 ($\pm$ NM)	-	Kattge et al. 2020
Leaf C/N	25.7 ($\pm$ 1.5)	23.9 ($\pm$ 4.6)	24.7 ($\pm$ 3.1)	Measured on green leaves from composite samples of 3 individuals per species (Thermo Fisher Scientific Flash 2000)	
Specific leaf area ($\text{cm}^2 \text{g}^{-1}$)	127.52 ($\pm$ 23.45)	121.30 ($\pm$ 33.74)	68.19 ($\pm$ 14.20)	-	Kattge et al. 2020
Aboveground biomass of herbaceous vegetation on tree row (dry biomass in g m^{-2})	1 632 ($\pm$ 928)	800 ($\pm$ 384)	928 ($\pm$ 432)	0.25 x 0.25 m quadrat placed between each studied tree pair	
Types of vegetation in tree rows (% coverage)	Grasses: 55 Dicotyledons: 32 Woody plants: 13 Bare soil: 0	Grasses: 84 Dicotyledons: 15 Woody plants: 1 Bare soil: 0	Grasses: 66 Dicotyledons: 31 Woody plants: 0 Bare soil: 3	Visual assessment of 5 tree pairs per species in the tree row (TR)	

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Soil function	Indicator name	Measured variable	Biological assemblages	Hypotheses	References
Soil structure maintenance	AggSurf	Aggregate stability (0-2 cm)	Macrofauna, fungi	Sensitivity to the position	Herrick et al. 2001
	AggSoil	Aggregate stability (2-10 cm)	Macrofauna, fungi		Herrick et al. 2001
	Beerkan	Infiltration rate	Soil engineers		Adapted from Lassabatère et al. 2006
	VESS	Soil structure assessment	Soil engineers		Guimarães, Ball and Tormena 2011
Nutrient cycle	AEMNO3	NO ₃ ⁻ fixed on ion exchange membranes	All microorganisms	Sensitivity to the species and position	Le Cadre et al. 2018; Qian et Schoenau 2002
	Nmin	Available N (NO ₃ ⁻ and NH ₄ ⁺)	All microorganisms		Maynard and Kalra, 1993
Carbon transformation	Lamina	Lamina bait	Mesofauna	Sensitivity to the species	von Törne 1990
	POXC	Permanganate Oxidizable Carbon	All microorganisms		Weil et al. 2003
	SituResp	Basal soil respiration	Microorganisms		Thoumazeau et al. 2017

Table 3. Environmental characteristics according to the positions and the tree species. Means ($\pm$ SD). The effects of the species and position factors were tested using a linear model. The test significance levels are indicated as follows: ns for $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Bold letter differences indicate significantly different results. Soil C/N, organic C and total N analyses were conducted on soil cores sampled at 0-10 cm depth. TR = tree row; C1 = crop alley 1; C2 = crop alley 2. ¹ p-values obtained by Kruskal-Wallis test

Environmental variable	Position effect					Tree species effect					Total
	TR	C1	C2	p-value	R ² contribution	Maple	Ash	Pear	p-value	R ² contribution	R ²
Temperature at 06:00 (°C) n = 3 per position and per species	17.5 ($\pm$ 1.8) b	17.6 ($\pm$ 1.8) b	20.0 ($\pm$ 1.8) a	***	88%	18.3 ($\pm$ 2.0)	19.0 ($\pm$ 2.2)	18.3 ($\pm$ 2.2)	*	12%	0.39
Temperature at 15:00 (°C)¹ n = 3 per position and per species	23.8 ($\pm$ 1.9) c	27.2 ($\pm$ 2.8) b	36.3 ($\pm$ 5.9) a	***	99%	31.6 ($\pm$ 6.3)	28.6 ($\pm$ 9.0)	28.1 ($\pm$ 6.2)	*	1%	0.68
Soil moisture (%) n = 5 per position and per species	23.3 ($\pm$ 4.7) a	18.8 ($\pm$ 2.7) b	16.4 ($\pm$ 3.9) b	***	100%	19.5 ($\pm$ 4.3)	19.3 ($\pm$ 3.9)	19.6 ($\pm$ 6.1)	ns	0%	0.35
Shade (%) n = 5 per position and per species	48.7 ($\pm$ 30.9)	43.2 ($\pm$ 34.5)	34.4 ($\pm$ 26.6)	ns	18%	27.1 ($\pm$ 29.3) b	58.0 ($\pm$ 22.1) a	41.3 ($\pm$ 33.2) ab	*	82%	0.21
Soil C/N n = 5 per position and per species	28.1($\pm$ 2.6) b	36.1($\pm$ 1.4) a	36.4($\pm$ 2.0) a	***	97%	32.6 ($\pm$ 4.0)	34.2 ($\pm$ 4.1)	33.9 ($\pm$ 5.1)	ns	3%	0.82
Soil organic C (%) n = 5 per position and per species	3.77 ($\pm$ 0.69) a	2.31 ($\pm$ 0.20) b	2.27($\pm$ 0.19) b	***	98%	2.83 ($\pm$ 0.69)	2.65 ($\pm$ 0.88)	2.89 ($\pm$ 0.89)	ns	2%	0.75
Soil total N (%) n = 5 per position and per species	0.37 ($\pm$ 0.04) a	0.25 ($\pm$ 0.01) b	0.25 ($\pm$ 0.02) b	***	98%	0.30 ($\pm$ 0.05) a	0.28 ($\pm$ 0.06) b	0.29 ($\pm$ 0.08) b	ns	2%	0.81
Aboveground biomass (g m⁻²) n = 5 per position and per species	1 616 ($\pm$ 768) a	688 ($\pm$ 336) b	1 024 ($\pm$ 672) b	***	55%	1 632 ($\pm$ 928) a	800 ($\pm$ 384) b	928 ($\pm$ 432) b	***	45%	0.59

Table 4. Summary of the Biofunctool® indicator results for the whole dataset (n = 45). The effects of species (n = 15 per tree species) and position factors (n = 15 per location) were tested using a linear model. The test significance levels are indicated as follows: ns for p > 0.05; *p < 0.05; **p < 0.01; ***p < 0.001.

Method	Soil function	Indicator	Mean	Minimum	Maximum	Position effects		Tree species effects		Total
						p-value	R ² contribution	p-value	R ² contribution	R ²
Biofunctool®	Soil structure maintenance	AggSurf (score)	4.0	0.8	6.0	***	91%	ns	9%	0.57
		AggSoil (score)	4.3	1.5	6.0	***	89%	ns	11%	0.41
		Beerkan (mL min ⁻¹)	1514	264	3734	***	99%	ns	1%	0.69
		VESS (score)	3.3	1.6	5	***	94%	ns	6%	0.42
	Nutrient cycling	AEMNO3 (µg N-NO ₃ ⁻ cm ⁻² d ⁻¹)	0.78	0.09	2.2	ns	45%	*	55%	0.24
		Nmin (mg N kg ⁻¹ soil)	0.43	0.04	2.31	**	85%	ns	15%	0.30
	Carbon transformation	Lamina (% deg d ⁻¹)	4.55	0.63	7.57	ns	8%	***	92%	0.35
		POXC (mg kg ⁻¹ soil)	744	418	1113	***	92%	*	8%	0.75
		SituResp (score)	0.40	0.03	1.00	***	99%	ns	1%	0.55

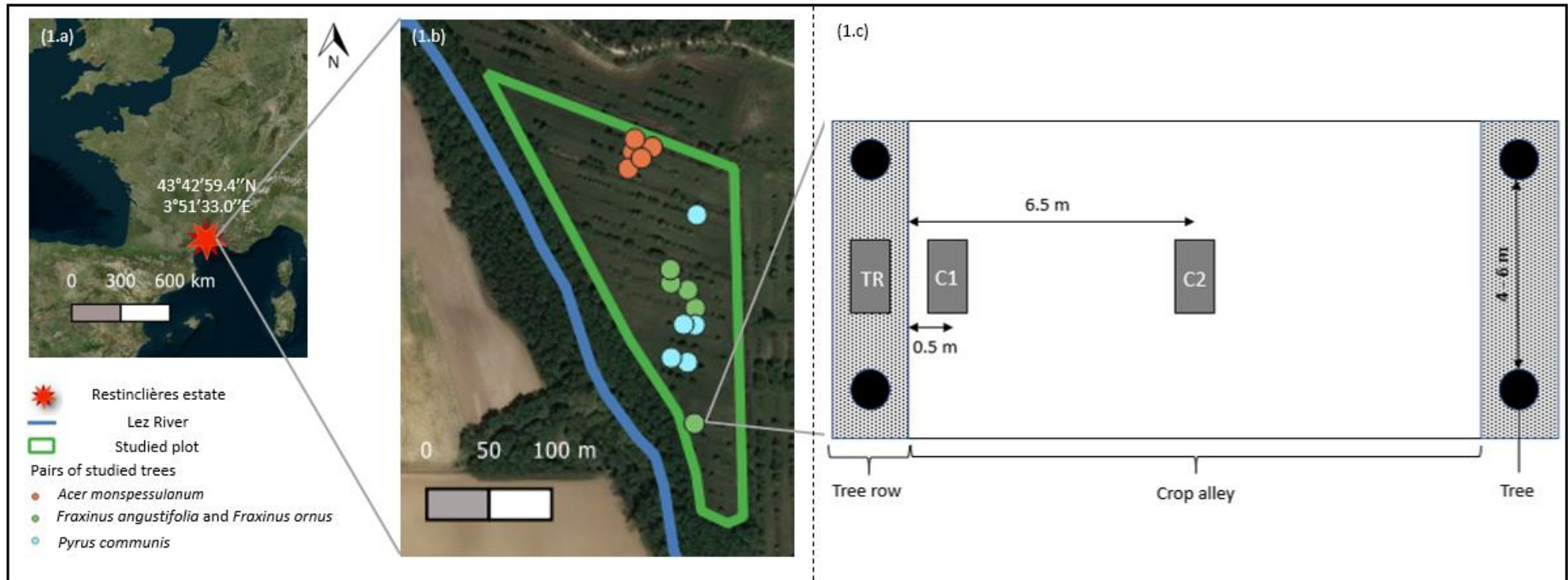


Figure 1. Location of the study (1.a), pairs of trees studied in the plot (1.b) and layout of the three sampling positions (TR, C1, C2) for a given tree pair.

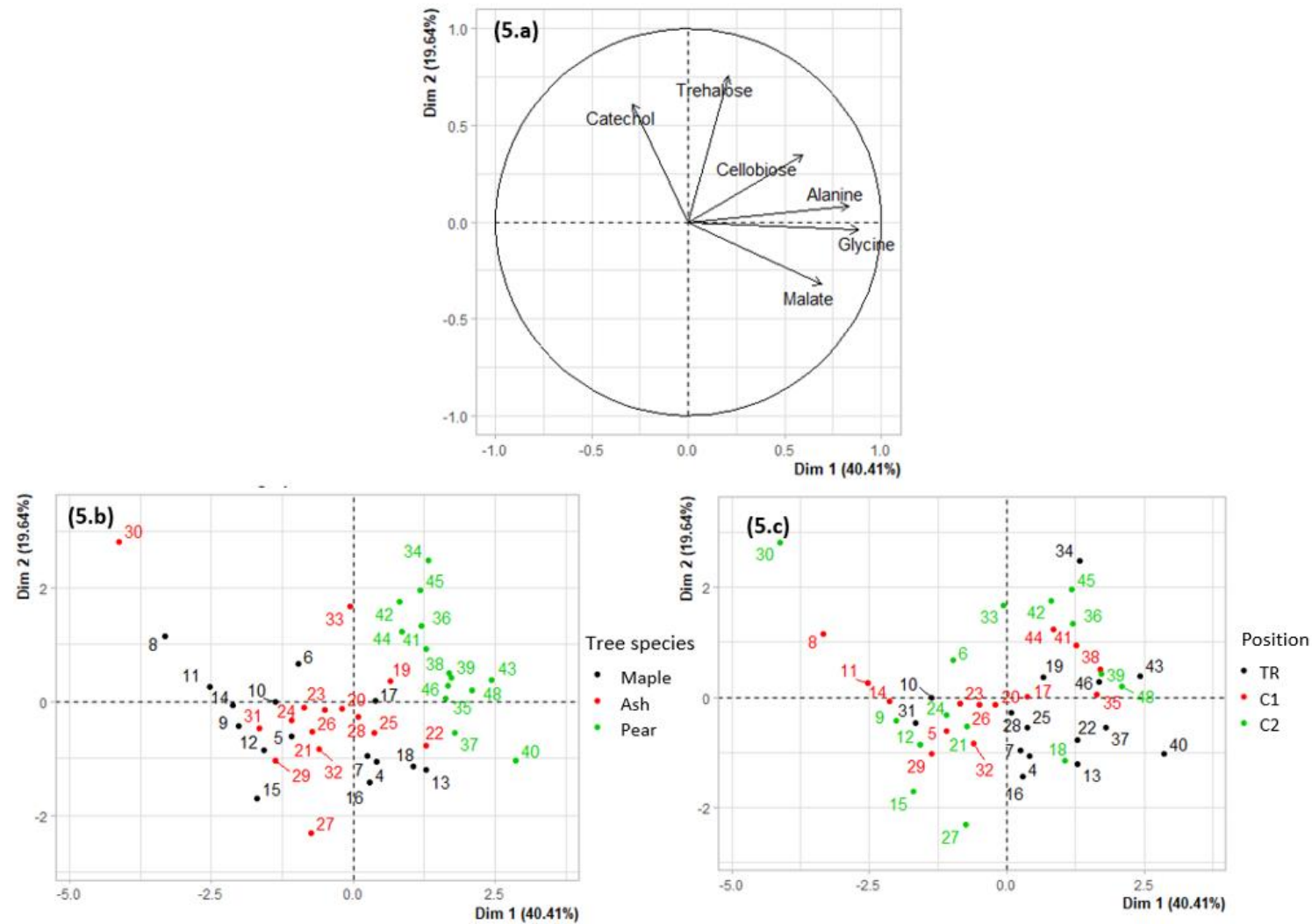


Figure 2. Principal component analysis performed on the Biofunctool® indicators for all 45 sampling points. (2.a) is the graph of variables on dimensions 1 and 2. (2.b) is the plot of individuals grouped according to the position factor on dimensions 1 and 2. (2.c) is the graph of individuals grouped according to the species factor on dimensions 1 and 2.

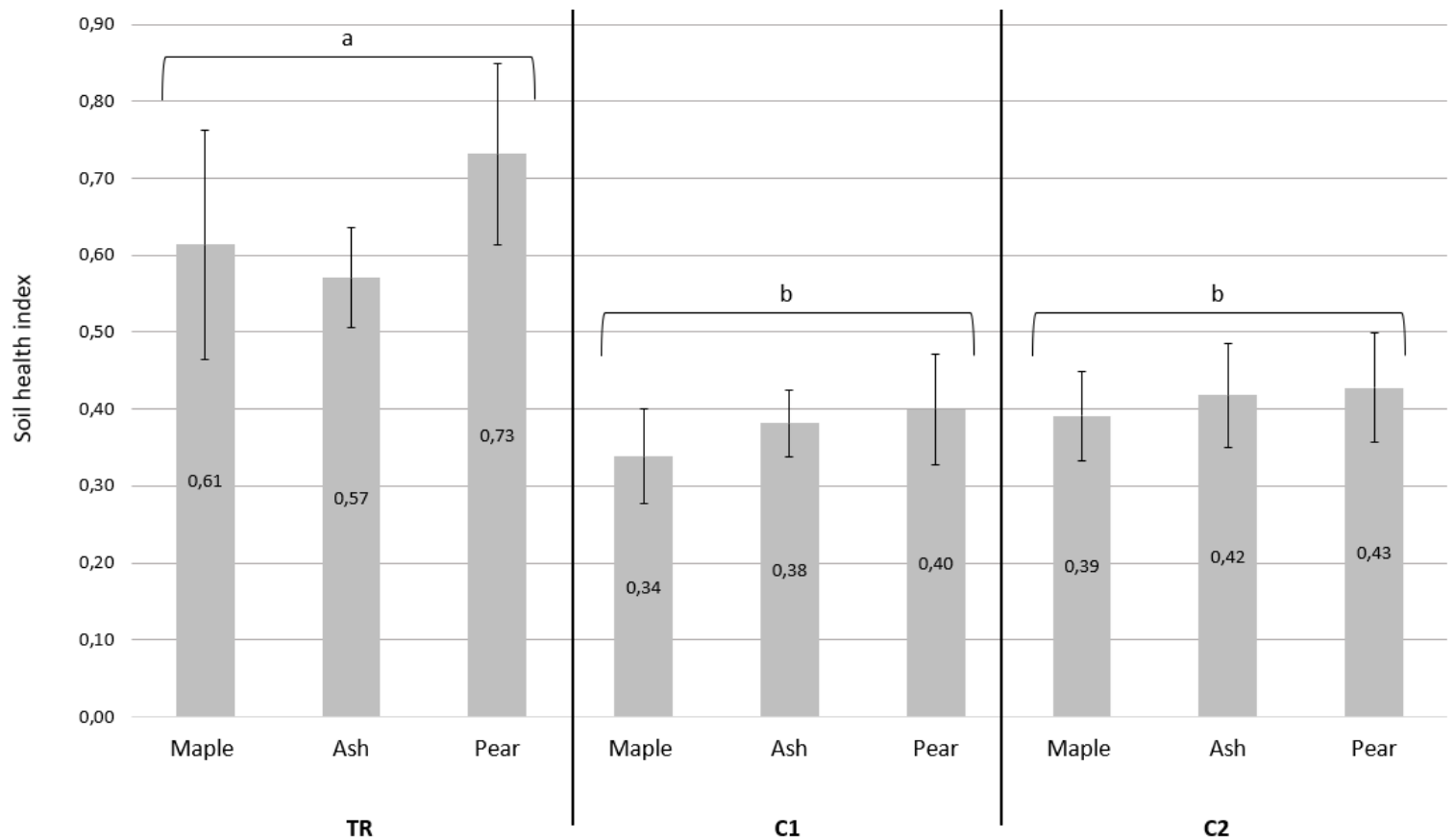


Figure 3. Soil health index after aggregation of the Biofunctool® indicator results. The index was drawn up according to the procedure described by Thoumazeau, Bessou, Renevier, Trap et al. (2019). The different letters indicate significant differences between positions (Tukey post-hoc test). n = 5 per treatment; mean ± SD.

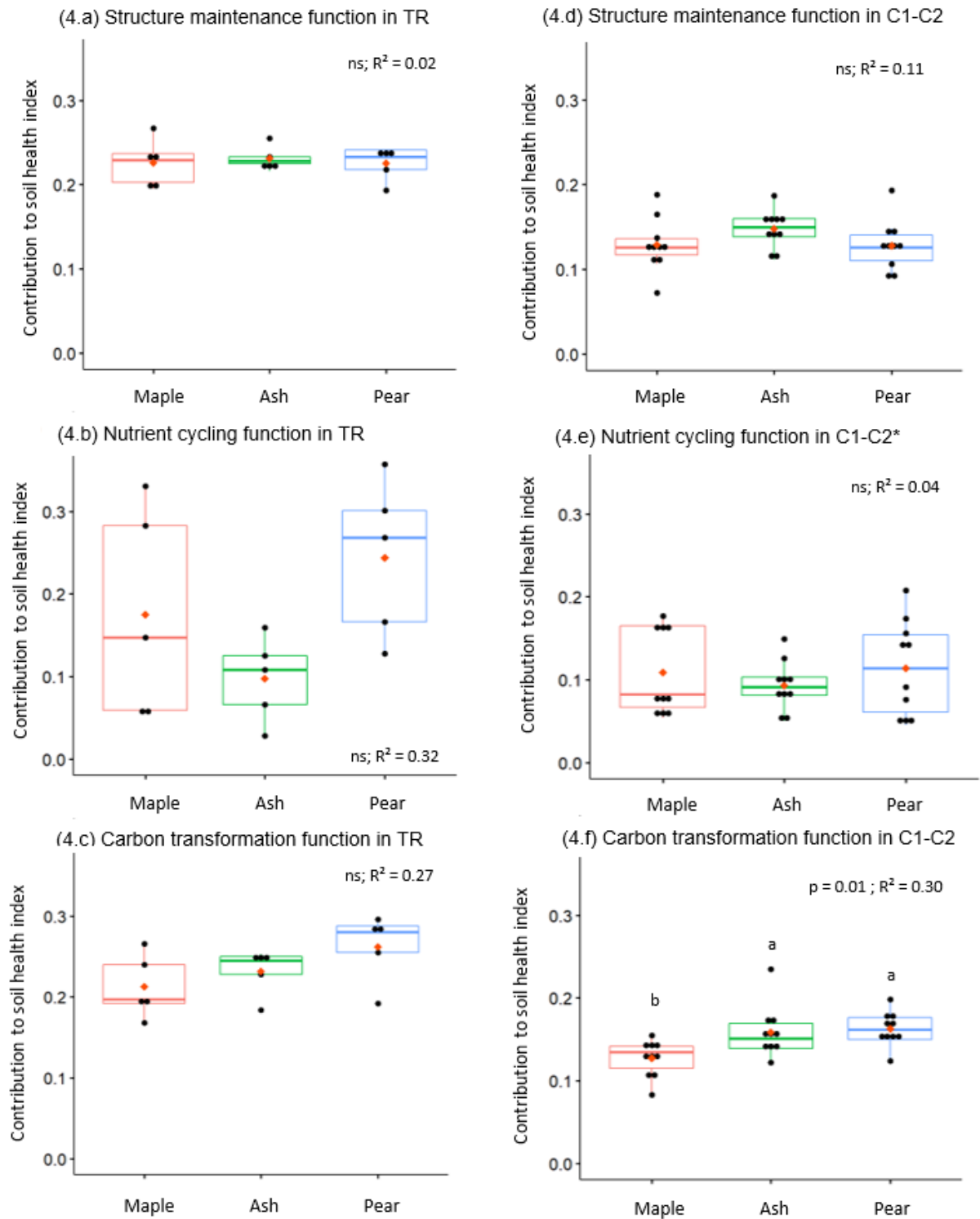


Figure 4. Effect of tree species on the three soil functions studied under tree rows (TR, graphs a, b, c) or in crop alleys (C1 and C2, graphs d, e, f). The three functions represent Biofunctool[®] indicator contributions to the total score presented in Figure 3. Structure maintenance = AggSurf + AggSoil + Beerkan + VESS; Nutrient cycling = AEMNO3 + Nmin; Carbon dynamics = Lamina + POXC + SituResp. Red diamonds represent means. * indicates when a Kruskal-Wallis test was performed, otherwise an analysis of variance followed by a Tukey test was performed. The different letters indicate significant differences (Tukey post-hoc test).

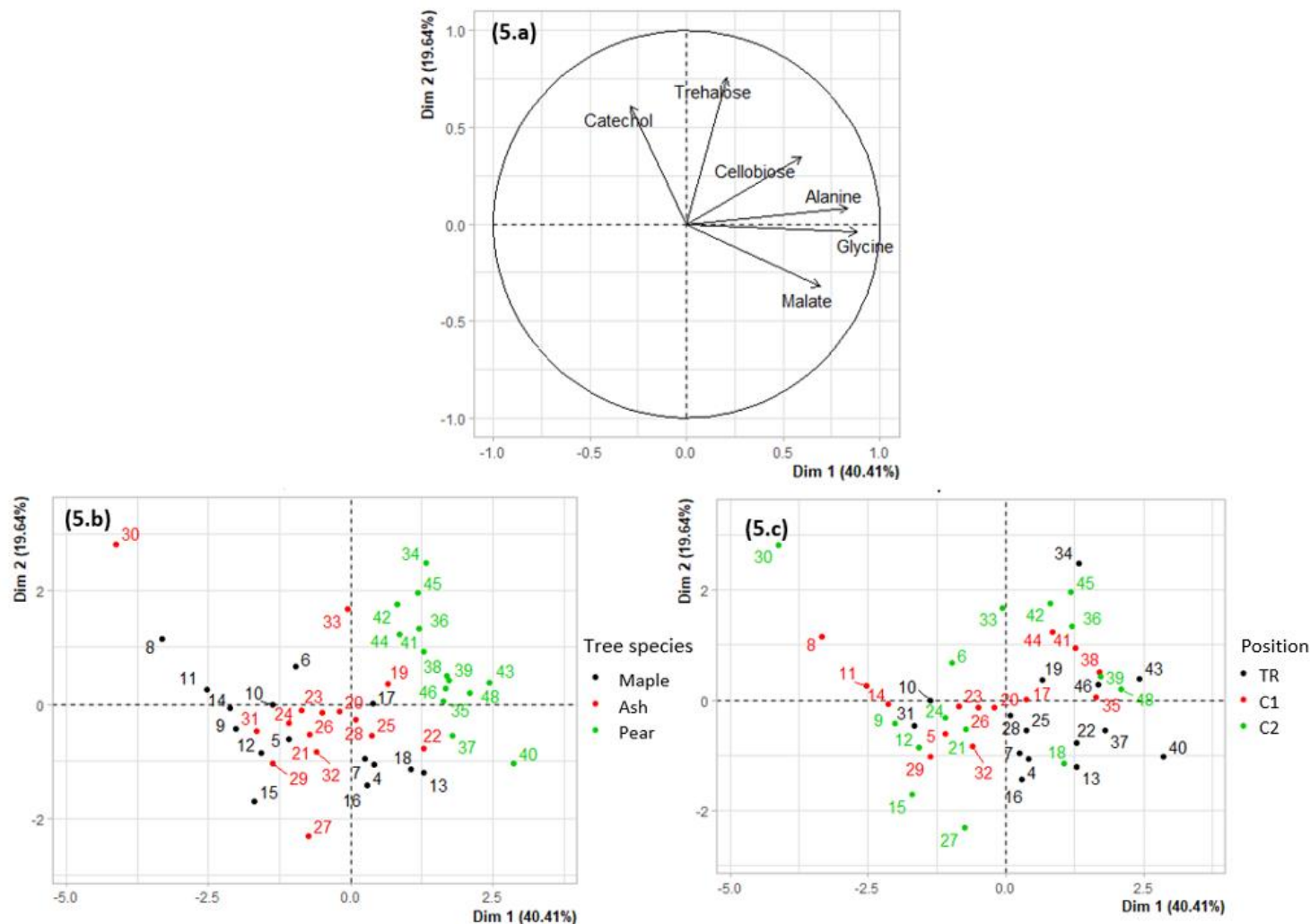


Figure 5. Principal component analysis performed on the MicroResp™ indicators for all 45 sampling points. (5.a) shows a graph of variables on dimensions 1 and 2. (5.b) shows a graph of individuals grouped by species on dimensions 1 and 2. (5.c) shows a graph of individuals grouped according to the position relative to the tree on dimensions 1 and 2. NB: a first PCA revealed an outlier (replicate 5, crop alley C1, pear tree species) which was not taken into account for this PCA.

Figure captions:

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