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Perennial crops shape the soil microbial community and increase the soil carbon in the upper soil layer

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ABSTRACT

Soil biodiversity is threatened by intensive agriculture that relies on annual grain crop production, thus leading to a decline in soil functions and ecosystem services. Perennial grain crops have a positive impact on the soil microbial community, but the responsive microbial groups and the magnitude of their response remain uncertain. To elucidate this, we analysed soil microbial biomass and community composition, bacterial growth and soil total carbon in five crops: organic perennial intermediate wheatgrass (IWG, *Thinopyrum intermedium*, Kernza®), organic IWG-alfalfa intercrop, organic biennial grass-legume mixture, organic annual wheat or rye and conventional annual wheat. The analysis was carried out at three time points under two growing seasons at four different soil depths. Five years after establishment, IWG had greater amounts of soil total fungi and bacteria, and of arbuscular mycorrhizal (AM) fungi, saprotrophic fungi, gram-negative (G^-) and gram-positive (G^+) bacteria compared to annual wheat. Crop perenniality influenced the soil microbial community structure although precipitation, soil temperature and water content were the main drivers of the patterns of and temporal variations in the microbial community. Perennial crops, with reduced tillage and low nitrogen input management increased the proportions of fungi relative to bacteria, AM fungi to saprotrophic fungi, G^- bacteria to G^+ bacteria, and the growth rate of total bacteria. This resulted in a more active soil microbial community with higher microbial biomass than annual wheat and contributed to the increased soil total carbon storage in the 0–5 cm soil layer in a humid continental climate. The findings emphasize the importance of combining a no tillage strategy with long-term vegetation cover to increase soil quality.

1. Introduction

Soil microbial organisms are vital for agricultural production and soil health due to their significant roles in nutrient cycling and other ecosystem processes (Barrios, 2007). For example, soil arbuscular mycorrhizal (AM) fungi form symbioses with most terrestrial plants and influence plant nutrient uptake (Jeffries et al., 2003). Soil saprotrophic fungi and bacteria decompose soil organic matter and influence nutrient availability for plants (Ning et al., 2021). Symbiotic bacteria such as *Rhizobium sp.* can form nodules with legume roots and fix nitrogen from the atmosphere (Soumare et al., 2020). Furthermore, the necromass of soil microbes contributes to 24%–60% of the soil organic carbon pool and contributes to soil carbon sequestration (Deng and Liang, 2022).

Soil microbiota differs among soils depending on factors such as land use and management. Intensive agriculture with monocultures of annual crops decreases soil microbial biomass (Yan et al., 2022), reduces soil biodiversity, simplifies soil food webs, and threatens overall soil functioning (Tsiafouli et al., 2015) due to the intensive management (*e.g.* soil tillage, re-sowing, fertilizer application, chemical and mechanical weed management). As a result, annual crop production is responsible for a range of environmental problems including soil degradation and other pollution (Kopittke et al., 2019). Thus, current conventional agricultural practices for producing annual crops negatively influence soil health (Yang et al., 2020) and undermine the possibility of long-term sustainability in crop production (Cárceles Rodríguez et al., 2022).

Perennial cereal grain crops can be productive for several years without the need to be re-sown every year, significantly reducing soil disturbance otherwise resulting from tillage (Chapman et al., 2022). The year-round presence of roots, combined with the inherent capacity to grow large root biomass (Sprunger et al., 2018), perennial crops

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minimize nitrate leaching (Jungers et al., 2019; Huddell et al., 2023) and emit root exudates into the soil ecosystem (Ma et al., 2022). All these factors combined result in a production system that increases soil organic matter (Kim et al., 2022), enhances water use efficiency (de Oliveira et al., 2018; Clément et al., 2022), improves soil structure (Daly et al., 2023), sustains soil fertility (Mosier et al., 2021), reduces soil erosion (Cosentino et al., 2015) and sequesters more carbon in the soil (Peixoto et al., 2022). The transition from annual to perennial cereal grain crops is therefore suggested as a way to enhance sustainability in agriculture (Duchene et al., 2020; Chapman et al., 2022; Zhang et al., 2023).

The first perennial cereal crop, intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey, Kernza®) or IWG, has been selected and domesticated for grain production and forage use (Wagoner and Schaeffer, 1990; Culman et al., 2013; Bajgain et al., 2022). In the past ten years, IWG has been evaluated for its grain (Dick et al., 2018; Fernandez et al., 2020; Hunter et al., 2020a) and biomass production (Jungers et al., 2017; Favre et al., 2019; Culman et al., 2023), environmental benefits (Culman et al., 2013; DeHaan and Ismail, 2017; Rakkar et al., 2023) and economic benefits (Hunter et al., 2020b; Law et al., 2022a) under various management practices in the United States and Europe. At current stage of perennial grain crop development, the grain yield of IWG (112–1212 kg ha^{-1}, Culman et al., 2013; Law et al., 2022b) is significantly lower than conventional annual wheat (global average 4020 kg ha^{-1}, Dadrasi et al., 2023). Replacing annual wheat with IWG at this stage would cause serious loss of food production. Meanwhile, the environmental benefits provided by IWG as a pioneer perennial crop has already been proven in many studies thus IWG was suggested for production on marginal land or incorporating in the crop rotation systems to increase crop diversity (Duchene et al., 2019, 2020).

Despite the numerous advantages of perennial grain crops on soil health, relatively few studies have been conducted on the complex plant-soil-microbe interaction. The extended lifetime of perennial grains causes a prolonged interaction with the soil microbiome as compared to annual crops. How the soil microbial community responds to the increased crop longevity (*i.e.* perenniality), undisturbed upper soil layers and sustained fibrous root growth is not yet fully understood. Recent studies have provided valuable insights into how IWG and annual wheat differ regarding soil microbial community composition (Sprunger et al., 2019; Duchene et al., 2020; Mckenna et al., 2020), microbial biomass (Audu et al., 2022; Rakkar et al., 2023; Taylor et al., 2024) and the related soil carbon storage capacity (Sprunger et al., 2019; Audu et al., 2022; Taylor et al., 2023), but they have not reached a consistent conclusion regarding which is the dominating microbial community. For example, Sprunger et al. (2019) reported that IWG increased bacterial and nematode diversity and richness under certain nitrogen fertilizer levels 4 years after planting IWG. Duchene et al. (2020) found that fungal abundance, but not bacterial abundance, increased in IWG cropping in the first and second spring seasons after planting. Rakkar et al. (2023) found that perennial cropping systems improved soil microbial biomass and other soil health parameters more than annual systems at the 0–15 cm soil depth. Audu et al. (2022) reported that two-year-old IWG systems had higher microbial biomass, microbial activities and soil organic carbon compared to annual wheat at the 30–60 cm soil depth but not the 0–30 cm soil depth.

Soil depth has a great impact on soil microbial communities because unequal plant roots distribution and resource availability tends to be higher at the soil surface and declines with depth (Hao et al., 2021). Soil microbial communities are also greatly influenced by seasonal dynamics (Kramer et al., 2013; Contosta et al., 2015) since temperature and moisture are important determinants of microbial activities (Brockett et al., 2012). Furthermore, the magnitude of the response of soil microbial communities and soil health improvements due to perennial grain crops depends on the specific soil type and local climate (Rakkar et al., 2023). With the growing interest in perennial grain agriculture in Europe, it is important to evaluate how perennial grain crop production influences soil microbial biomass, communities, and soil parameters in the Scandinavian climate.

The aim of this study was to better understand how crop perenniality affects soil microbial biomass and communities. More specifically, the objectives were to investigate whether longer-duration perennial crops increase microbial diversity and abundance, and whether prolonged root development in perennial crops and reduced soil disturbance significantly contribute to enhanced soil carbon storage. Therefore, we compared soil microbial community composition, biomass, bacterial growth, and soil total carbon with regard to organic perennial cropping (IWG sole crop, IWG-alfalfa intercropping), organic biennial ley cropping, and organic and conventional annual cropping (wheat or rye) for two growing seasons at a 0–40 cm soil depth. We hypothesised that crops with high levels of perenniality would host a more diverse and more abundant soil microbial community and accumulate more carbon relative to crops with low levels of perenniality.

2. Material and methods

2.1. Experimental site

The experiment was conducted at the Swedish Infrastructure for Ecosystem Science's (SITES) Agroecological Field Experiment (SAFE) field located at the SITES Lönnstorp Research Station, SLU, in Alnarp (55.65° N, 13.06° E) in a region with a humid continental climate (Fig. 1). The mean annual precipitation (from 2015 to 2022) was 533 mm and the daily average temperature was 10 °C. The soil type is a sandy loam soil with 67% sand and 18% clay; the basic physical and chemical properties of the soil were reported by Li et al. (2020). The SAFE was established in 2016 and is replicated in four fully randomized blocks with each cropping system represented in every block. Within SAFE, five crops were studied: organic perennial intermediate wheatgrass (IWG, Thinopyrum intermedium, Kernza®), organic IWG-alfalfa intercrop, organic biennial grass-legume mixture, organic annual wheat or rye, and conventional annual wheat.

2.2. Crop selection and sampling

To study the effects of crop perenniality on soil microbes and carbon, we selected five crops from three SAFE crop system components based on their different lifespan: (1) organic IWG sole crop (perenniality level five), (2) organic IWG-alfalfa intercropping (perenniality level five) (3) ley from the organic rotation (perenniality level two), (4) rye or wheat from the organic rotation (perenniality level one), and (5) wheat from the conventional rotation (perenniality level one). To study the seasonal

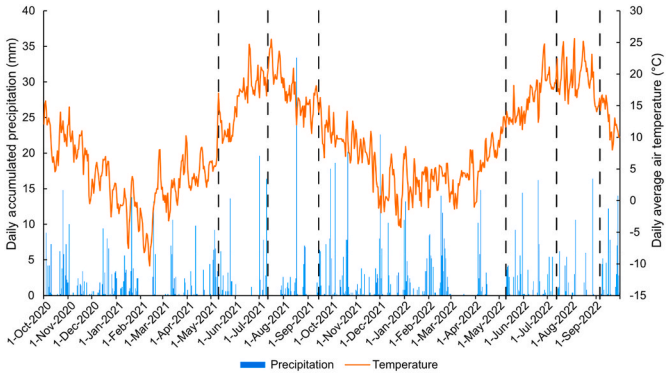

Fig. 1. The daily accumulated precipitation (mm) and average daily air (20 cm aboveground) temperature from October 1, 2020 to September 30, 2022. Dashed vertical lines indicate the sampling dates. The precipitation and air temperature data were collected by the in situ automatic weather station at the SITES Lönnstorp Research Station and retrieved from the SITES data portal (https://data.fieldsites.se/portal/).

dynamics of soil microbes, soil samples were collected at the beginning, peak and end of crop growing season; *i.e.* May, July and September in Sweden, as well as for two consecutive years (2021, 2022) for improving the reliability and repeatability of the results. Rye was part of the SAFE organic rotation system in 2021, but it was rotated out and replaced by wheat in 2022; therefore, the rye field was sampled in 2021 while the wheat field was sampled in 2022. No sampling in ley in May 2021 due to planning defects.

The IWG seeds were accessed from the Cycle 3 germplasm of the perennial grain breeding program at The Land Institute of Salinas, Kansas, USA (Zhang et al., 2016). Both IWG and IWG-alfalfa intercrops were sown with a row spacing of 25 cm, and the IWG-alfalfa intercrops were sown in separate alternate rows. The biennial crop ley is a legume-grass mixture which consists of 15% tall fescue (*Festuca arundinacea*), 10% red clover (*Trifolium pretense*), 5% white clover (*Trifolium repens*), 20% alfalfa (*Medicago sativa*), 30% timothy (*Phleum pratense*) and 20% ryegrass (*Lolium*). The planting and field management activities from 2016 to 2019 for perennial cropping systems have been previously described in detail (Audu et al., 2022; Dimitrova Mårtensson et al., 2022). The agronomic management activities, which include sowing, fertilizing, and herbicide or pesticide application in all cropping systems, are summarized in Table S1.

The plant material was sampled from one duplicate subplot (0.25 m^2) in each experimental plot at four randomized blocks on May 10, 2021, 12–13 July 2021, 14–15 September 2021, May 9, 2022, 12–13 July 2022, 5–6 September 2022 (*i.e.* five and six years after IWG planting). After collecting plant samples, soil samples were collected with a soil auger (2.5 cm diameter) at four corners of each sub-plot at 0–5, 5–15, 15–30, and 30–40 cm soil layers. The four soil augers were thoroughly mixed to obtain a composite sample per plot at each soil layer. After sampling, the soil samples were stored immediately in cool boxes and transported to the laboratory. The soil samples were sieved (through 2 mm) for homogenization within 24 h. A 50 g subsample of soil was stored in a −20 °C freezer and freeze-dried later for the microbial analyses. A 200 g subsample of soil was air-dried for pH, total carbon and nitrogen concentration analyses, and another 100 g subsample of soil was stored at 4 °C for soil bacterial growth rate and soil water content analyses.

2.3. Microbial abundance and structure

The phospholipid fatty acid (PLFA) from cell membranes varies in carbon chain length, saturation and branching in different microorganisms thus can be used as biomarkers for microbial community structure and metabolic activity in environmental studies (Willers et al., 2015). The PLFA method was chosen for the current study because PLFAs degrade rapidly after cell death and can identify living microbial biomass and is more sensitive in detecting shifts in the microbial community compared to DNA/RNA based methods, although it cannot provide detailed species composition or phylogenetic resolution when used on its own (Ramsey et al., 2006; Willers et al., 2015). Genetic analysis was not carried out in current study.

The PLFA and neutral lipids fatty acid (NLFA) analyses are based on the single-phase extraction of lipids described by Bligh and Dyer (1959) and Frostegård et al. (1993). The lipids were extracted from 2 g freeze-dried soil samples in a chloroform:methanol:citrate buffer mixture (1:2:0.8 v/v/v, pH = 4). The soil sample and mixture were vortexed and extracted at room temperature for 2 h. Then the extracts were split into two phases by adding chloroform and citrate buffer (pH = 4), and the phase containing lipid was dried under a stream of nitrogen gas. The lipid material was fractionated on a pre-packed silica column (Agilent Bond Elut, LRC, 10 ml, 40 μm) into neutral lipids, glycolipids and phospholipids by eluting with chloroform, acetone and methanol, respectively. An internal standard methyl nonadecanoate (19:0) was added to the phospholipid and neutral lipid fractions for fatty acid quantification. The samples were then methylated using a mild alkaline methanolysis to produce fatty acid methyl esters, which were then separated and quantified by gas chromatograph (polar column) with a flame ionisation detector (GC-17A, Shimadzu). The peak identification of different PLFAs and NLFAs were based on the retention times of external fatty acid methyl ester standards. Future studies should add a known amount of phospholipid, such as di19:0 PC, in the soil to serve as a recovery standard prior to extraction for quality control.

In total, 26 different fatty acids were identified in this study based on the relative retention time, and 13 of them were considered to be of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, 17:1ω8, i17:0, a17.0, 17:0, cy17:0, 18:1ω7, and cy19:0); the sum of these 13 PLFA was used as an indicator of total bacteria biomass (Barreiro et al., 2015). Three fatty acids were identified and considered to be of fungal origin: PLFA 18:2ω6 is an indicator for saprotrophic fungi, PLFA 18:1ω9 is a general fungal indicator and PLFA 16:1ω5 indicates AM fungi in the soil; the sum of these 3 PLFAs was used as an index of fungal biomass (Kaiser et al., 2010; Barreiro et al., 2022). Both PLFA 16:1ω5 and NLFA 16:1ω5 were used as biomarkers for AM fungi (Lehman et al., 2012; Vestberg et al., 2012; Kundel et al., 2020). The ratio between NLFA 16:1ω5 and PLFA 16:1ω5 was used to indicate the structure and abundance of AM fungi in the soil (Olsson et al., 1997; Vestberg et al., 2012). Furthermore, the G^- bacteria was indicated by PLFAs 16:1ω7c, cy17:0, 18:1ω7, and cy19:0, G^+ bacteria was indicated by PLFAs i14:0, i15:0, i16:0, and 10Me18, and Actinobacteria was indicated by 10Me16, 10Me17, and 10Me18.

The total PLFA concentration was used as an indicator for total microbial biomass in soil, and soil microbial biomass carbon was estimated by multiplying the total PLFA concentration by a factor of 5.8 (Joergensen and Emmerling, 2006). The microbial biomass was calculated per gram of organic matter (Frostegård and Bååth, 1996). The ratio between fungi indicator PLFA abundance and bacterial indicator PLFA abundance was used to indicate the proportion of fungi relative to bacteria in the microbial community. The ratio between PLFA16:1ω5 and PLFA 18:2ω6 was used to indicate the proportion of AM fungi relative to saprotrophic fungi, and the ratio between G^- bacteria and G^+ bacteria was used to indicate the proportion of G^- bacteria relative to G^+ bacteria.

2.4. Bacterial activity

Bacterial activity is indicated by growth rate (*i.e.* protein synthesis rate), which was estimated by the incorporation of radiolabelled leucine into protein (Bååth, 1994; Söderberg and Bååth, 1998; Bååth et al., 2001). In brief, 1 g of fresh soil was added to 20 ml of distilled water to create the bacteria suspension, and then 1.5 ml of the bacterial suspension was incubated with 2 μl ^{3}H-labelled leucine (1 mCi 37 MBq, PerkinElmer) for 2 h at room temperature. The incubation was terminated by adding 75 μl 100% Trichloroacetic acid. After centrifugation and the removal of the supernatant, the pellet was washed with 1.5 ml 80% ethanol and 1.5 ml 5% trichloroacetic acid. The pellet was re-suspended in 200 μl 1 M NaOH and heated at 90 °C for 1 h. A 1 ml scintillator cocktail (Sigma-Aldrich, United Kingdom) was then added and the radioactivity was counted in a scintillation counter (Hidex 300 SL).

2.5. Soil physico-chemical properties

The soil total carbon and nitrogen concentration were analysed by a FLASH 2000 Organic Elemental Analyzer (Thermo Scientific) using air-dried and milled (<1 mm) soil samples. Soil organic matter (SOM) concentration was determined as soil loss on ignition at 550 °C for 3 h (Hoogsteen et al., 2015) and calculated according to Moebius-Clune et al. (2016). Soil organic carbon (SOC) concentration was estimated from soil organic matter using an SOC-to-SOM conversion factor of 0.58 g SOC/g SOM (Heikkinen et al., 2021). Apparent soil bulk density was estimated from the soil organic carbon concentration using pedotransfer

functions (Kätterer et al., 2006). Soil total carbon mass per unit soil area was calculated as the product of soil total carbon concentration, apparent soil bulk density, and soil thickness at fixed soil depths (Ellert et al., 2001). Soil water content was calculated as the percentage of fresh soil weight loss after drying in an oven at 105 °C for 24 h. Soil temperature was monitored by soil temperature and moisture sensors (CS655, Campbell Scientific) installed 10 cm deep in the experimental fields from 10 May to July 20, 2021 and from 16 May to September 23, 2022. The soil temperature data for September 14, 2021 and May 9, 2022 were obtained from the weather station at the research station.

2.6. Statistical analysis

Multivariate analyses were used to show the patterns of PLFA data and to analyse with explanatory variables. The relative abundance of individual PLFAs (the percentage of each fatty acid's peak area within the total fatty acid's peak area) was used to coordinate data for multivariate analyses. The constrained ordination method called Partial Redundancy Analysis (pRDA) was used to explain the variation in the soil PLFA profile due to environmental variables after accounting for the variation explained by blocks. Stepwise Regression Forward Selection (based on 999 permutations) was conducted in R to select the relevant explanatory variables, and Holm correction was used to correct the significance level. Based on the forward selection, only significant (adjusted $p < 0.05$, Holm correction) constrained explanatory variables were included in the pRDA model ($p < 0.001$). Nine explanatory variables—precipitation, soil depth, sampling time (May 2021, July 2021, and September 2022), crop perenniality, soil water content, soil temperature and soil total carbon concentration—were selected by forward selection based on the level of significance to conduct the pRDA. The explanatory variable precipitation was defined as the accumulated rainfall during the two weeks prior to the sampling dates. The explanatory variable soil temperature was defined as the daily average soil temperature on the sampling day. The significance of each explanatory variable was tested individually and shown to be significant ($p = 0.026$ each). The first six canonical axes resulting from the pRDA were also statistically significant ($p = 0.001$). The significance of the differences between different cropping systems was tested by PERMANOVA analysis, the ADONIS test and multilevel pairwise comparison using the vegan package (Oksanen et al., 2013).

A randomized complete block design was selected for the experiment, with three treatment factors—cropping system, soil depth and sampling time—and repeated measurements of experiment units (block: cropping systems interaction) at different sampling times. The effects of cropping system, soil depth, sampling time and their interactions on the estimated microbial biomass and ratios, bacterial growth, total microbial biomass carbon and soil total carbon (concentration and mass) were tested by analysis of variance (ANOVA). A three-way ANOVA (Type III analysis of variance with Kenward-Roger's method) was used to test the effects of cropping system, soil depth and sampling time for the complete and balanced data set consisting of perennial IWG sole cropping, perennial IWG-alfalfa intercropping, biennial ley cropping and conventional annual wheat cropping at all soil depths during the sampling times of July 2021, September 2021, May 2022, July 2022, and September 2022. A two-way ANOVA was used to test the effects of cropping system and soil depth for the complete and balanced data set in May 2021. Specifically, the effects of the treatment were fitted in a linear mixed-effects model and analysed by ANOVA. Models were created using the "lme4" package, with cropping system, soil depth, and sampling time treated as fixed effects for the three-way ANOVA (or cropping system and soil depth treated as fixed effects for the two-way ANOVA). The blocks and experiment units (block:cropping systems interaction) are treated as random effects since the observations from the same experiment unit may be correlated. The outliers in AM fungi to saprotrophic fungi ratio data were replaced by the median values of other replicates. Assumptions of normally distributed residual errors and homogeneity of variance were checked by plotting residuals against fitted values and QQ plots. Box-Cox transformation was used for all the parameters to fulfil the assumptions of normality. The estimated marginal means of these variables calculated with the "emmeans" package were reported as treatment means. Pairwise comparisons of means were conducted with Tukey's method adjustment for multiple comparisons. All the statistical tests use $\alpha = 0.05$ as the significance level of effects. The Kendall correlation was used to analyse the correlation between soil microbial biomass carbon and soil total carbon concentration. All analyses were performed using R statistical software (R studio, version 4.2.0).

3. Results

3.1. Soil microbial community composition and main drivers

The pRDA model (adjusted $R^2 = 0.47$, $p = 0.001$) shows that 48.1% of the variation in soil PLFA profiles can be explained by the constrained explanatory variables of precipitation, soil depth, sampling time, crop perenniality, soil water content, soil temperature and soil total carbon concentration, with their magnitude of influence (indicated by the increases of adjusted R^2) corresponding to the order presented above (Fig. 2). Furthermore, 1.59% of the variation can be explained by the conditioned variable (block), while 50.3% of the variation was left unexplained.

Most of the variation was aggregated on the first constrained axis RDA1 (32.6% of the variance) along which soil PLFAs were associated with either high soil temperature, high precipitation and high soil water

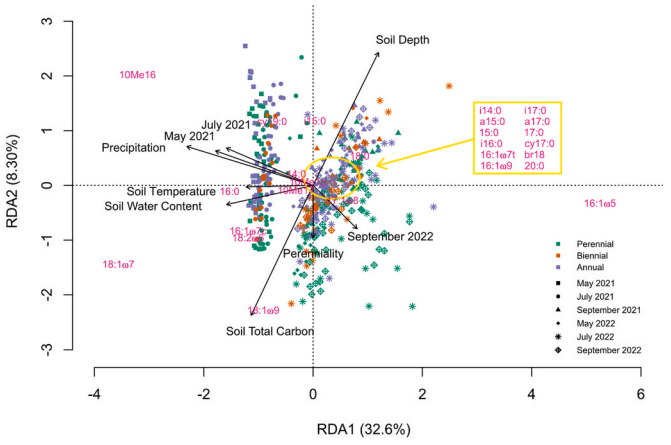

Fig. 2. Partial redundancy analysis (pRDA) of soil microbial PLFA profiles in 2021 and 2022. The name of fatty acids consist of total numbe of carbon atoms: number of double bounds, followed by the position of the double bound from the methyl end of the molecule. Cis and trans configurations are indicated by c and t. The prefixes a and i indicate anteiso- and iso-branching; br indicates unknown methyl branching position; cy indicated to cyclopropane fatty acids; and 10Me indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule. Colours indicate crop perenniality: green indicates perennial crops that were five years old including both intermediate wheatgrass sole crop (IWG) and the intercrop of IWG with alfalfa, orange indicates biennial crops that were two years old including only ley crop, and purple indicates annual crops that were one year old including organic winter wheat, rye and conventional winter wheat. Symbols indicate sampling time points, with squares indicating May 2021, circles July 2021, triangles September 2021, rhombuses May 2022, stars July 2022 and diamonds with plus sign September 2022. Arrows indicate the direction in which the gradient of the environmental variable was greatest, and the length of arrow indicates the strength of the correlation between the environmental variables and the microbes. Only significant variables based on stepwise regression forward selection ($p < 0.05$; based on 999 permutations) are displayed. The first constrained axis RDA1 and the second constrained axis RDA2 have the largest eigenvalues, and their contribution to the variance is the largest.

content in May and July 2021 (negative RDA1 scores), or low soil temperature, low precipitation, and low water content in September 2022 (positive RDA1 scores) (Fig. 2). Statistically, the main effect of the explanatory variables for May 2021 ($F = 80.5$, $p = 0.001$), July 2021 ($F = 127$, $p = 0.001$) and September 2022 ($F = 15.6$, $p = 0.001$) on soil microbial PLFAs was significant, while the main effect of the explanatory variables for September 2021, May 2022 and July 2021 was non-significant (according to the forward selection). Therefore, the latter three explanatory variables were not presented as arrows in the pRDA plot (Fig. 2).

The soil microbial community in May 2021 was significantly different from that of July 2021 and September 2022 (Pairwise Comparison, $p = 0.001$). The soil microbial community in July 2021 was also significantly different from that of September 2022 (Pairwise Comparison, $p = 0.001$). Specifically, the PLFA indicators for Actinobacteria (10Me16, 10Me17, 10Me18), G^- bacteria (16:1ω7c, 18:1ω7, cy19:0), saprotrophic fungi (18:2ω6) and general bacteria (16:0) were more abundant under high soil temperature and high precipitation conditions of May and July 2021 (Fig. 2); *i.e.* respectively, 37.8 and 49.6 mm accumulated rainfall in two weeks prior to sampling, 17.0 and 20.7 °C average air temperature (Figs. 1), and 23.6 and 17.9 °C average soil temperatures on the sampling days (May 10 and July 12, 2021). On the other hand, the PLFA indicators for AM fungi (16:1ω5), general bacteria (15:0, 16:1ω7t, 16:1ω9, a17:0, i17:0, 17:1ω8) and G^+ bacteria (i14:0, i16:0) were more abundant under low soil temperature and low precipitation conditions of September 2022 (Fig. 2); *i.e.* 16.6 mm accumulated rainfall in two weeks prior to sampling, 16.2 °C average air temperature (Figs. 1), and 19.5 °C average soil temperature on the sampling day (September 5, 2022).

The crop perenniality gradients associated with the second constrained axis RDA2 (8.30% of the variance) differentiated the perennial crops, which were characterized by a higher soil total carbon concentration in the upper soil layers (negative RDA2 scores), from the annual crops, which were characterized by a lower soil total carbon concentration in the deeper soil layers (positive RDA2 scores) (Fig. 2). Statistically, the main effect of crop perenniality on the soil microbial community composition was significant (PERMANOVA, Adonis2, $F = 18.3$, $p = 0.001$). There were separations of soil microbial PLFAs among the centres of three crop perenniality clusters (Fig. S1). The soil microbial community under perennial crops was significantly different from that under annual crops (Pairwise Comparison, $p = 0.001$) and biennial crops (Pairwise Comparison, $p = 0.005$). The soil microbial community under biennial crops was also significantly different from annual crops (Pairwise Comparison, $p = 0.03$). Specifically, the PLFA indicators for general fungi (18:1ω9), saprotrophic fungi (18:2ω6), G^- bacteria (16:1ω7c, 18:1ω7) and AM fungi (16:1ω5) were more abundant when there was a higher degree of perenniality and higher soil total carbon, while PLFA indicators for G^+ bacterial (i15:0), G^- bacteria (cy19:0), and Actinobacteria (10Me16) were more abundant when there was a lower degree of perenniality and low soil total carbon. Overall, both fungal (18:2ω6, 18:1ω9, 16:1ω5) and bacterial (*e.g.* 16:1ω7c, 18:1ω7) PLFA indicators were abundant in perennial and biennial cropping systems, while only bacterial (*e.g.* 15:0, i17:0, i15:0, cy19:0) PLFA indicators were abundant in annual wheat cropping, indicating a shift in soil microbial community composition towards a more fungi-abundant community with perennial cropping.

Soil depth also contributed to the variations associated with the RDA2 axis, and it influences the pattern of soil microbial community ordination significantly (PERMANOVA, Adonis2, $F = 23.0$, $p = 0.001$) (Fig. 2). Different soil depths had significantly different soil microbial community compositions (Pairwise Comparison, $p = 0.001$). The PLFA indicators for saprotrophic fungi (18:2ω6), fungi (18:1ω9), G^- bacteria (18:1ω7, 16:1ω7c), and Actinobacteria (10Me17) were more abundant in the wet upper soil layers 0–5 cm and 5–15 cm (Fig. 2) with high soil water content (10.6% and 10.0%, respectively). The PLFA indicators for general bacteria (*e.g.* 16:1ω9, a17:0, a15:0, 15:0, 17:0) and G^+ bacteria (i14:0, i15:0, i16:0) were more abundant in the dry lower soil layers at 15–30 cm and 30–40 cm with low soil water content (8.90% and 7.85%, respectively).

3.2. Microbial biomass, structure and activity in different cropping systems

3.2.1. Perennial IWG sole crop vs. conventional annual wheat

Crops with different levels of perenniality had different amounts of soil total microbes, total fungi, total bacteria, AM fungi, saprotrophic fungi, G^- bacteria, G^+ bacteria, and Actinobacteria, as well as a different fungi:bacteria ratio, AM fungi:saprotrophic fungi ratio, and G^- bacteria:G^+ bacteria ratio; the magnitude of these differences depended on the soil depth and sampling time point (Fig. 3; Fig. 4, Table S2, Table S3). The high perenniality IWG sole crop had significantly higher ($p < 0.05$) total PLFA biomass than low perenniality conventional annual wheat at a soil depth of 0–30 cm in May 2021, 0–40 cm in May 2022, 0–15 cm in July 2021 and July 2022, 0–5 cm in September 2021, and 5–15 cm in September 2022 (Fig. S2). The IWG sole crop had significantly ($p < 0.05$) higher estimated AM fungi (NLFA 16:1ω5) biomass than conventional wheat at almost all soil depths and sampling time points except for September 2022, during which it was observed only in lower soil layers at 15–40 cm (Fig. 3). The estimated saprotrophic fungi (PLFA 18:2ω6) biomass was higher as well under the perennial IWG sole crop ($p < 0.05$) than under conventional annual wheat at all soil depths in May 2021 (Fig. 3), while in July 2021, May 2022 and July 2022, it was mainly higher in the upper soil layer at 0–5 cm. For total fungi, total bacteria and G^- bacteria, the biomass were significantly higher ($p < 0.05$) in the IWG sole crop than the conventional annual wheat crop mainly in the upper soil layers at 0–5 cm and/or 5–15 cm except for May 2021 and May 2022, during which the values of these microbial group indicators were higher in IWG at almost all soil depths (Fig. 3). Similarly, the amount of G^+ bacteria was significantly higher ($p < 0.05$) in the perennial IWG sole crop than in the conventional annual wheat crop in the upper soil layers, specifically at 0–15 cm in May 2021 and July 2021 and at 0–5 cm and 15–30 cm in May 2022 (Fig. 3). On the other hand, Actinobacteria were more abundant in the perennial IWG sole crop than in the conventional annual wheat crop but in the deeper layers at 5–30 cm in May 2021, 5–15 cm in July 2021 and 30–40 cm in July 2022 (Fig. 3).

Regarding bacterial growth, the perennial IWG sole crop had significantly higher ($p < 0.05$) values than the conventional annual wheat crop at almost all soil depths in May 2021, July 2021 and September 2021, and in shallow soil layers 0–5 cm in May 2022 and in 15–30 cm in September 2022 (Fig. 5). The perennial IWG sole crop had a significantly higher ($p < 0.05$) microbial biomass carbon concentration than conventional annual wheat crop at 0–30 cm in May 2021 and July 2021, 0–15 cm in September 2021, July 2021 and September 2022, and 0–5 cm in May 2022 (Fig. 6).

Finally, the different ratios were also significantly higher ($p < 0.05$) for the perennial IWG sole crop compared with the annual wheat at different soil depths. The fungi:bacteria ratio was higher at soil depths of 0–5 cm and 30–40 cm in May 2021, 30–40 cm in July 2021 and May 2022, and 15–30 cm in September 2022 (Fig. 4). The AM fungi:saprotrophic fungi ratio was higher at a lower soil depth of 15–40 cm in May 2021 and July 2022, and at almost all soil depths in July 2021 and September 2022 (Fig. 4). The G^- bacteria:G^+ bacteria ratio was higher at 0–15 cm in May 2021 and at almost all depths in July 2022 (Fig. 4).

3.2.2. Perennial IWG sole crop vs. biennial ley crop

The differences in the estimated biomass of AM fungi (NLFA 16:1ω5), saprotrophic fungi (PLFA 18:2ω6), total fungi, total bacteria, G^- bacteria, G^+ bacteria, and Actinobacteria, as well as the fungi:bacteria ratio, AM fungi:saprotrophic fungi ratio, and G^- bacteria:G^+ bacteria ratio between the perennial IWG sole crop and biennial ley crop were not significant in most combinations of soil depths and sampling time points

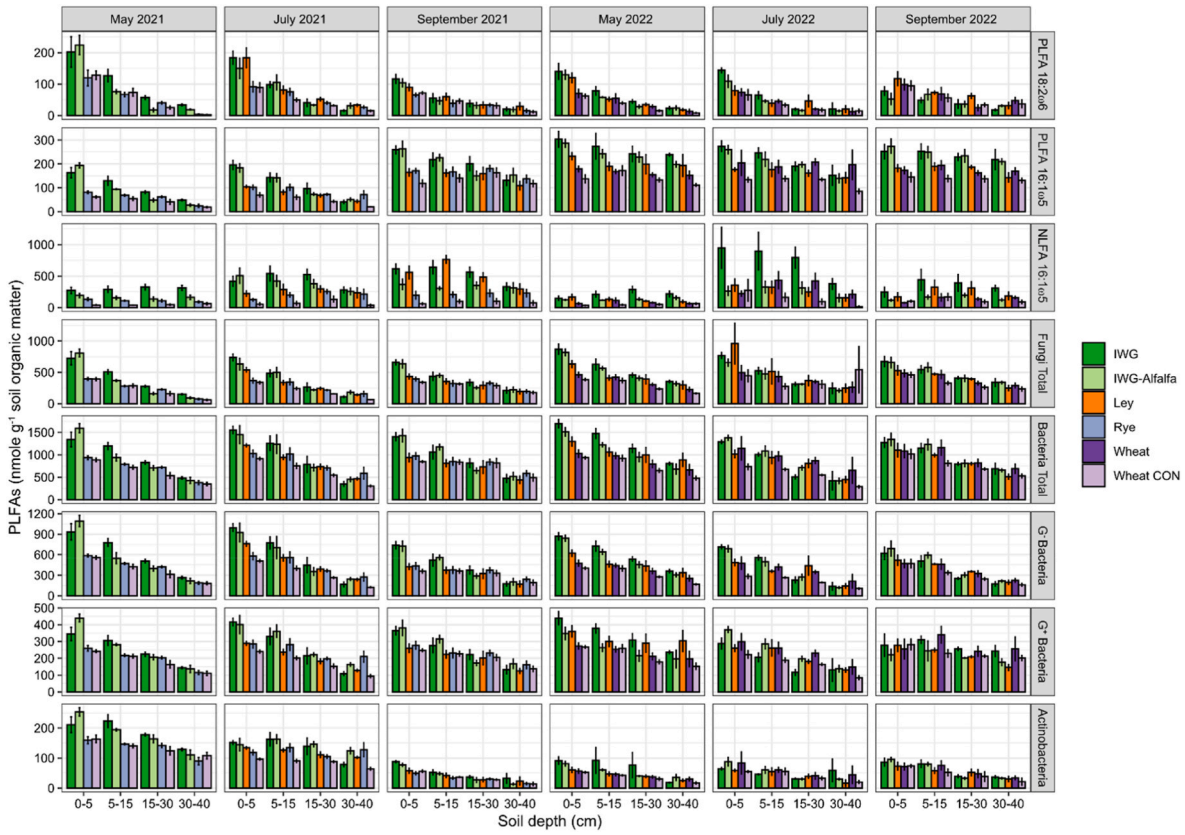

Fig. 3. Quantification of soil microbial PLFA profiles at four soil depths in 2021 and 2022. Colours indicate a specific crop: dark green indicates the perennial Intermediate Wheatgrass (IWG) sole crop under organic management, light green indicates intercropped IWG with alfalfa under organic management, orange indicates the biennial ley crop under organic management, light blue indicates annual rye under organic management, dark purple indicates annual wheat under organic management, and light purple indicates annual wheat under conventional (CON) management.

(Fig. 3; Fig 4, Table S2, Table S3). In several circumstances, the high perenniality IWG sole crop had a significantly higher ($p < 0.05$) estimated biomass of these microbial groups than the lower perenniality biennial ley crop. For example, the perennial IWG sole crop had a higher ($p < 0.05$) estimated AM fungi (NLFA 16:1ω5) biomass than the biennial ley crop at all soil depths in July 2022 (Fig. 3), a higher ($p < 0.05$) estimated saprotrophic fungi (PLFA 18:2ω6) biomass at 0–5 cm in July 2022, a higher ($p < 0.05$) estimated total bacterial biomass and G^- bacterial biomass at 0–5 cm in September 2021 and at 5–15 cm in May 2022, and a higher G^+ bacterial biomass at 30–40 cm in September 2022 (Fig. 3). However, the perennial IWG sole crop had a significantly ($p < 0.05$) lower estimated total bacterial biomass and G^- bacterial biomass than the biennial ley crop at 15–30 cm in July 2022 (Fig. 3), and a lower soil total carbon concentration and mass at 15–30 cm in September 2022 (Fig. 6; Fig. S3).

Bacterial growth was higher ($p < 0.05$) for the perennial IWG sole crop than the biennial ley crop at all soil depths in September 2022 and at 0–5 cm in May 2022 (Fig. 5). The perennial IWG sole crop had a higher ($p < 0.05$) total microbial biomass than the biennial ley crop only at 5–15 cm in May 2022 (Fig. S2), a higher ($p < 0.05$) microbial biomass carbon concentration at 0–5 cm in July 2021, September 2021 and September 2022 (Fig. 6), and a higher ($p < 0.05$) soil total carbon concentration at 0–5 cm at all sampling time points (Fig. 6).

The ratios were generally higher for the perennial IWG sole crop than the biennial ley crop. The fungi:bacteria ratio was higher at 30–40 cm in May 2022 and at 5–30 cm in July 2022 (Fig. 4). The AM fungi:saprotrophic fungi ratio was higher at 15–40 cm in July 2021 and July 2022, and at 0–5 cm in September 2022 (Fig. 4). The G^- bacteria:G^+ bacteria ratio was higher at 5–15 cm in July 2022, but lower at 15–40 cm in September 2022 (Fig. 4).

3.2.3. *Biennial ley crop vs. conventional annual wheat crop*

The biennial ley crop had a similar amount of soil total microbes, total fungi, total bacteria, AM fungi, saprotrophic fungi, G^- bacteria, G^+ bacteria, and Actinobacteria, and a similar fungi:bacteria ratio, AM fungi:saprotrophic fungi ratio, and G^- bacteria:G^+ bacteria ratio compared to the conventional annual wheat crop in most combinations of soil depths and sampling time points (Fig. 3; Fig. 4, Table S2, Table S3). Under several conditions, the biennial ley crop had a significantly higher ($p < 0.05$) biomass of these microbial groups than the conventional annual wheat crop. For example, the biennial ley crop had a higher ($p < 0.05$) amount of AM fungi (NLFA 16:1ω5) than conventional annual wheat crop at almost all soil depths in July 2021 and September 2021 (Fig. 3). The biennial ley crop had a higher ($p < 0.05$) saprotrophic fungal (PLFA 18:2ω6) and total fungi biomass than conventional annual wheat at 0–5 cm in May 2022 and at 30–40 cm in July 2021 and May 2022 (Fig. 3). The amounts of total bacteria and G^+ bacteria were higher for the ley crop than the conventional annual wheat crop at 15–40 cm in May 2022, while the amount of G^- bacteria was higher at 0–5 cm and 30–40 cm in May 2022, and at 0–5 cm and 15–30 cm in July 2022 (Fig. 3).

Bacterial growth was less for the biennial ley crop than the conventional annual wheat crop, but only at 0–5 cm in September 2022 (Fig. 5). The biennial ley crop had a higher ($p < 0.05$) total microbial biomass than the conventional annual wheat crop at 0–5 cm in July 2022 (Fig. S2), a higher microbial biomass carbon concentration at 0–5 cm in July 2022 and at 0–5 and 15–30 cm in July 2021 (Fig. 6), and a higher soil total carbon concentration at 15–30 cm in September 2022 (Fig. 6).

For the ratios, the biennial ley crop had a higher ($p < 0.05$) fungi: bacteria ratio than the conventional annual wheat crop at 30–40 cm in

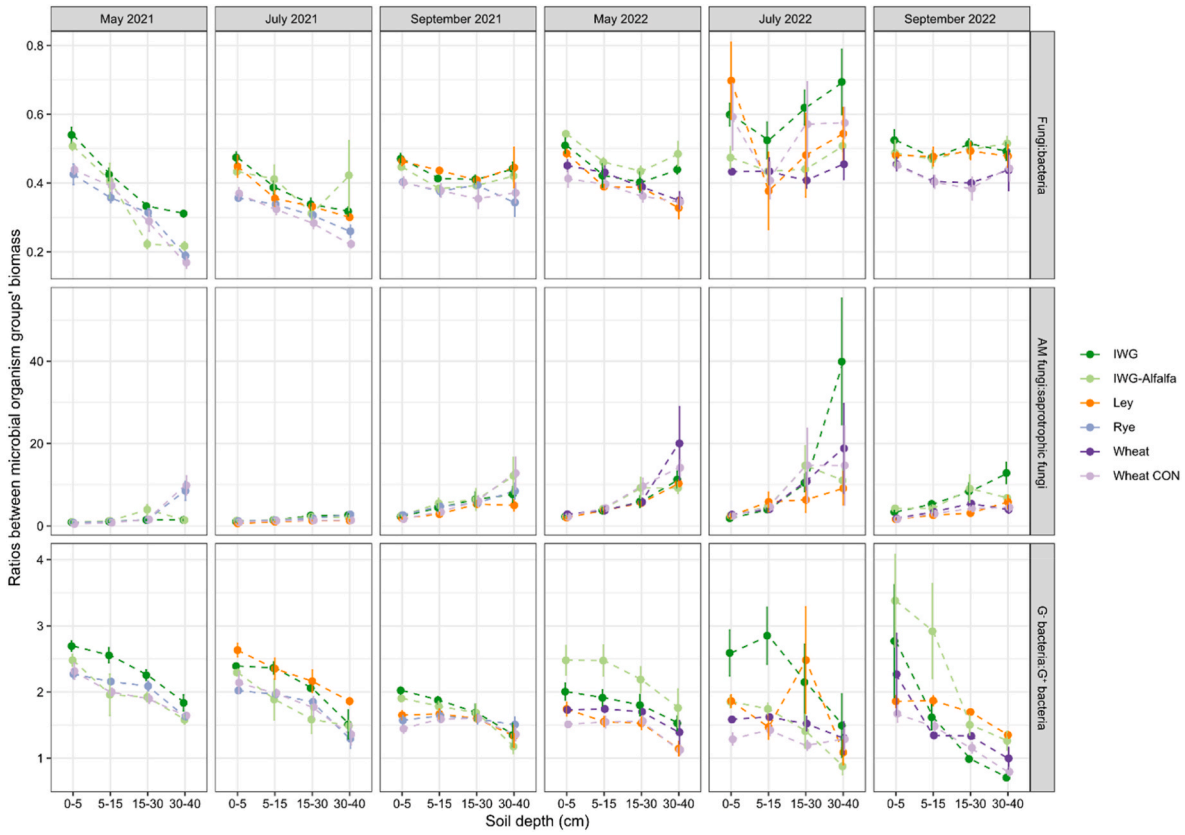

Fig. 4. Ratios of total fungi to total bacteria, arbuscular mycorrhizal (AM) fungi to saprotrophic fungi, and gram negative (G^-) to gram positive (G^+) bacteria at four soil depths in 2021 and 2022. Colours indicate a specific crop: dark green indicates the perennial intermediate wheatgrass (IWG) sole crop under organic management, light green indicates intercropped IWG with alfalfa under organic management, orange indicates the biennial ley crop under organic management, light blue indicates annual rye under organic management, dark purple indicates annual wheat under organic management, and light purple indicates annual wheat under conventional (CON) management.

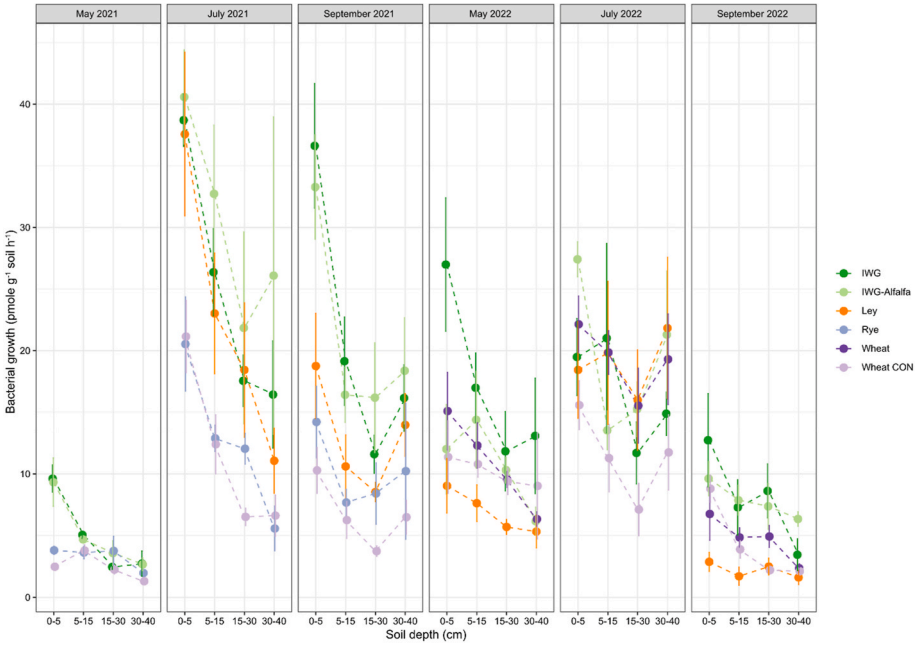

Fig. 5. Bacteria growth at four soil depths in 2021 and 2022. Colours indicate a specific crop: dark green indicates the perennial intermediate wheatgrass (IWG) sole crop under organic management, light green indicates intercropped IWG with alfalfa under organic management, orange indicates the biennial ley crop under organic management, light blue indicates annual rye under organic management, dark purple indicates annual wheat under organic management, and light purple indicates annual wheat under conventional (CON) management.

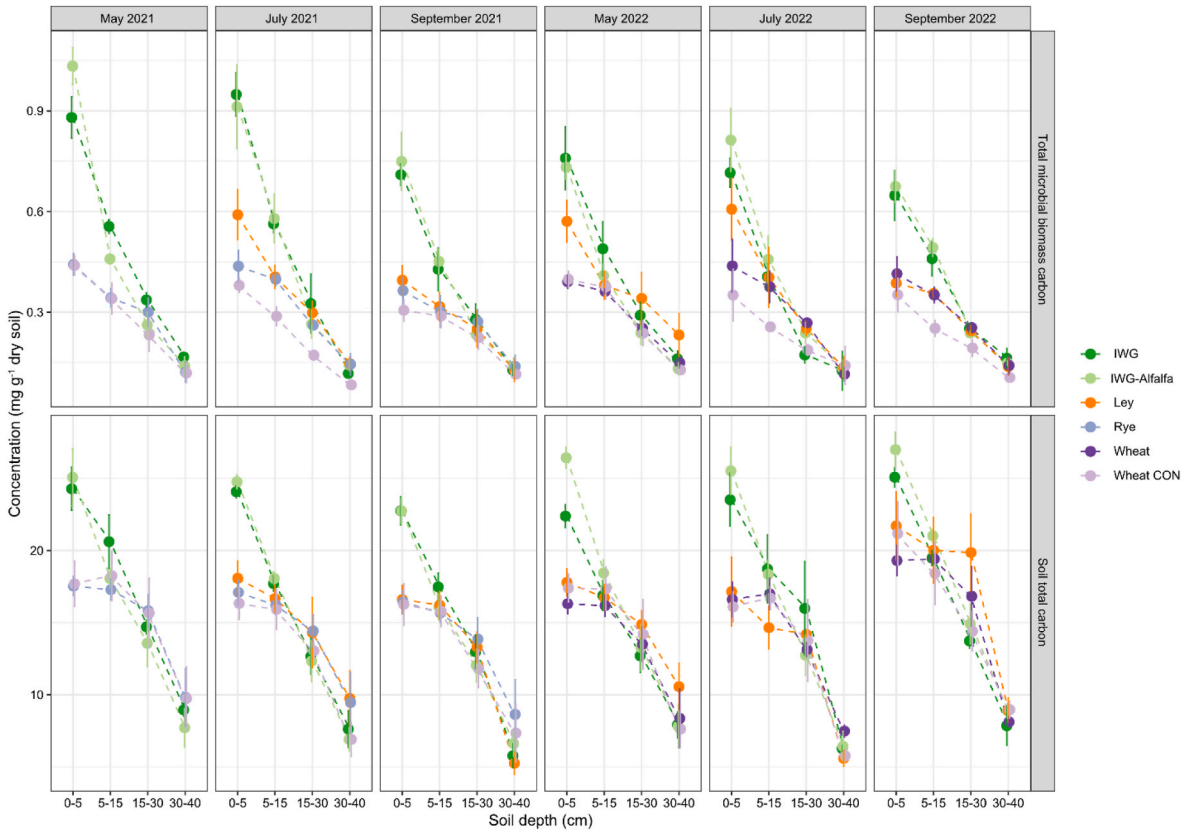

Fig. 6. Soil total microbial biomass carbon concentration and soil total carbon concentration at four soil depths at four soil depths in 2021 and 2022. Colours indicate a specific crop: dark green indicates the perennial intermediate wheatgrass (IWG) sole crop under organic management, light green indicates intercropped IWG with alfalfa under organic management, orange indicates the biennial ley crop under organic management, light blue indicates annual rye under organic management, dark purple indicates annual wheat under organic management, and light purple indicates annual wheat under conventional (CON) management.

July 2021 and a higher G^- bacteria:G^+ bacteria ratio at 15–30 cm in July 2022 and at 30–40 cm in September 2022 (Fig. 4).

Overall, IWG sole crop had the highest estimated biomass and ratios of microbial groups, conventional annual wheat had the lowest amount, and biennial ley had the medium amount in most combinations of soil depths and sampling time points. The differences among these three cropping systems reflected the trend of microbial biomass and ratios response to the gradients of crop perenniality. The microbial biomass and ratios of IWG-alfalfa intercropping were usually in the middle of that of IWG sole crop and biennial ley, and there was no significant difference ($p > 0.05$) among them in most circumstance. Under few conditions, such as at 5–40 cm in September 2022, IWG-alfalfa intercropping had higher G^- bacteria:G^+ bacteria ratio than IWG sole crop. IWG-alfalfa intercropping also had higher ($p < 0.05$) bacterial growth than biennial ley at almost all depths in September 2022. The soil microbial biomass and ratios of organic rye or wheat were in the middle of that of biennial ley and conventional annual wheat and the difference among them were not statistically significant ($p > 0.05$) in the most circumstance.

3.3. Correlation between microbial biomass carbon and soil total carbon

The soil total carbon concentration was positively correlated with crop perenniality and significantly contributes to the variation of PLFA patterns (Fig. 2). The soil total carbon concentration was higher ($p < 0.05$) in perennial IWG sole cropping and IWG-alfalfa intercropping than in conventional annual wheat cropping at 0–5 cm, and it was also higher ($p < 0.05$) in the upper soil layers compared to the lower soil layers (Fig. 6). The soil total carbon mass was higher ($p < 0.05$) in IWG-alfalfa intercropping than in conventional wheat cropping at 0–5 cm in July 2021, May and July 2022 (Fig. S3). Soil microbial biomass carbon was also higher ($p < 0.05$) in perennial IWG sole cropping than in conventional annual wheat cropping at 0–15 cm, and it was also higher ($p < 0.05$) in the upper soil layers compared to the lower soil layers (Fig. 6). The soil total carbon concentration was positively correlated with soil total microbial biomass carbon ($R^2 = 0.65$, $p < 0.001$) (Fig. S4).

4. Discussion

4.1. Soil microbial community structures shaped by climate-related variables associated with different seasons and soil depths

Climate-related variables (precipitation, soil water content and soil temperature) were found to be important factors affecting the temporal variations in soil microbial community composition and structure. The total amounts of fungi and AM fungi were more abundant in the microbial community under relatively dry and cold soil conditions compared to those under wet and warm conditions (illustrated in Fig. 2), which is in agreement with a comparative study of soil microbial communities under eight land-use types at a larger regional scale (Drenovsky et al., 2010). In this larger scale study, the soil water availability of different land use types was an important factor in structuring soil microbial communities; G^+, sulphate-reducing, anaerobic and general bacterial fatty acids were more abundant in wetter soils, while fungal and G^- bacterial fatty acids were more abundant in drier soils (Drenovsky et al., 2010). A similar pattern of results was obtained in a phylum level study by Castro et al. (2010), who reported that changes in precipitation had a significant impact on bacterial and fungal abundance; specifically, the relative abundance of Proteobacteria was greater in the wet treatments relative to the dry treatments, and Acidobacteria

abundance was greater in the dry treatments. Fungi were usually more abundant under dry soil conditions because fungi have a number of traits that can mitigate drought stress and help fungi survive and even grow under dry conditions (Treseder et al., 2010). For example, fungal chitinous cell walls protect fungal cell against the osmotic pressure inside the cell thus maintaining cellular shape (Cortés et al., 2019). Fungal hyphae can be produced slowly but consistently during a dry season enabling fungi to access water and nutrients (Treseder et al., 2010). Fungal mycelia networks can redistribute water along gradients in soil water potential (Guhr et al., 2015). The G^+ bacteria is more abundant under dry soil conditions than G^-, because G^+ bacteria have a thick and interlinked peptidoglycan cell wall which can act as protection towards water stress, while G^- bacteria have a single-layer cell wall and an outer membrane, making them generally more susceptible to water stress (Schimel et al., 2007). Some G^+ bacteria have the ability to form spores, which have the capability to take on a dormant cellular form and thus endure extreme conditions (*e.g.* drought) of their habitat (Andryukov et al., 2020).

The greater total fungal abundance (estimated by PLFAs abundance) found in upper soil layers in our study aligns with the results reported by Mckenna et al. (2020), signifying that the overall richness of fungal operational taxonomic units (OTU) and pathotroph OTU was higher at 0–10 cm than 10–30 cm for IWG sole cropping, annual cropping and native prairie. In our study, the upper soil layer was more enriched in fungi and G^- bacteria while the deeper soil layer was more enriched in general bacteria and G^+ bacteria. This is likely because fungi and G^- bacteria are responsible for decomposing the fresh plant-derived carbon accumulated in the upper soil layer, whereas G^+ bacteria are more capable of using recalcitrant compounds (Kramer and Gleixner, 2006; Tavi et al., 2013), and can assimilate carbon from dead fungal or root biomass that isn't directly from rhizodeposits (Tavi et al., 2013) at deeper soil layers. The saprotrophic fungi were more abundant in the upper soil layer for litter decomposition due to their specific enzymatic activities and high density of hyphae, as reported in other studies (Crowther et al., 2012; Guhr et al., 2015). We see a saprotrophic-to-mycorrhizal shift in fungal composition with increasing soil depth across different cropping systems and sampling times, which is in accordance with the general theory that the litter layer is generally dominated by saprotrophic fungi, while older and deeper layers are increasingly dominated by mycorrhizal fungi (Lindahl et al., 2007; Kyaschenko et al., 2017; Carteron et al., 2021). In this study, these changes in the microbial community's composition were largely driven by differences in soil moisture, water content and soil total carbon concentration, which were higher in the upper soil layers and lower in the deeper soil layers. Nevertheless, half of these variations in soil microbial community still remains unexplained by our model when all significant and measured environmental variables haven been considered. We speculate that part of the unexplained variations may attribute to the unknown environmental variables that were not measured in our study, such as soil inorganic nitrogen, available phosphorus, C:N:P stoichiometry, soil respiration, nutrient mineralization rates, fungal growth rates, root dynamics etc. Nutrient availability, microbial respiration and nutrient mineralization rates could give higher resolution in soil carbon and nitrogen dynamics which can be well correlated with soil microbial community composition shift and microbial activity.

We see a general decrease in the total fungal, total bacterial, AM fungal, and saprotrophic fungal biomass as soil depth increases, and this trend was more pronounced in the perennial cropping systems than in the annual cropping systems. This is likely because the nutrient stratification (*i.e.* variations with soil depth) becomes even more pronounced in perennial cropping due to the lack of tillage compared to annual cropping systems that are tilled frequently. Soil nutrient stratification provides a nutrient-rich environment that supports increased microbial biomass near the soil surface (Helgason et al., 2009). Greater soil nutrient stratification has been found in no-tillage systems due to the accumulation of crop residues and nutrients remaining immobile on the soil surface (Lupwayi et al., 2006). In annual cropping systems, however, the soil bacterial communities can be homogenized by tillage dispersal (West et al., 2023).

4.2. AM fungi-abundant microbial community with higher microbial biomass and activity under perennial cropping systems

The level of crop perenniality plays an important role in shaping the microbial community structure in agricultural soil. According to this study, the higher fungi:bacteria ratio in the perennial cropping systems compared to conventional annual wheat cropping systems in lower soil layers aligns with the results reported by Taylor et al. (2023), which showed that IWG had a higher fungi:bacteria ratio than tilled annual wheat at lower soil layers (15–60 cm). The higher total fungal and AM fungal biomass that our study found in perennial cropping systems compared to annual wheat cropping systems is also in line with the results reported by Duchene et al. (2020), which showed that overall fungi and AM fungi abundance increased in IWG cropping in the topsoil (0–10 cm) compared to annual cropping. The higher fungi proportion and abundance that appeared in cropping systems with high perenniality in our study can be explained by a combination of factors; for example, perennial crops provided long-term (5 yr) soil cover and root exudate input in the absence of tillage practices and low nitrogen fertilizer input.

Our results agree with earlier findings in which long-term land cover together with no-tillage practices had been found to increase fungal biomass (Helgason et al., 2009), fungal diversity (Schmidt et al., 2019) and the fungi:bacteria ratio (Sun et al., 2016) due to reduced physical disturbance and disruption of fungal hyphal network development. Soils with low nitrogen availability generally have fungal-based microbial communities and energy channels (Wardle et al., 2004; de Vries and Bardgett, 2012), whereas nitrogen-rich systems have bacterial-based microbial communities and energy channels, which follows the general assumption that nitrogen demand is predicted by the biomass C:N ratio (~5–15 in fungi compared to ~3–6 in bacteria) (Strickland and Rousk, 2010; Koranda et al., 2014).

The root quality and quantity of the perennial grain crop have been shown to shape soil food webs, and the C:N ratio of IWG coarse root is nearly twice that of annual wheat (Sprunger et al., 2019), suggesting more recalcitrant root tissue input in the soil (Duchene et al., 2020). This facilitates the colonization of fungi more than bacteria because fungi are capable of decomposing more recalcitrant substrates with higher C:N ratios (Hunt et al., 1987; de Vries et al., 2011). Perennial crops host a higher proportion of fungi relative to bacteria compared to annual crops, thus indicating that perennial and biennial cropping have fungal-based food webs while annual cropping has bacterial-based food webs. Except for the drought-resistant potential, which we discussed above in 4.1, fungal-based food webs are generally assumed to benefit other microbial communities since fungi provide assimilable (low molecular weight) substrates and nutrients to the whole microbial community (Beare et al., 1992).

According to this study, the average estimated AM fungi biomass (NLFA 16:1ω5) in perennial IWG sole cropping, which was 6.5 times that of annual wheat cropping, was comparable to the 5-times-greater AM fungal abundance reported by Duchene et al. (2020) in two-year-old IWG cropping compared to the annual rye cropping in France. The higher AM fungi biomass found in our study is attributable to the lack of disturbance of mycorrhizal fungi hyphae by tillage in the perennial cropping systems coupled with the sustained year-round root persistence that continually supplies the carbon resources. Additionally, the low organic nitrogen input in the perennial cropping system may induce and enhance the symbiotic relationship between AM fungi and IWG roots, thereby facilitating plant nutrient acquisition and water uptake. We observed a greater dominance of AM fungi over saprotrophic fungi in perennial IWG sole cropping compared to conventional annual wheat cropping. Furthermore, the AM fungi's dominance was more striking at the deeper soil depths in July and September 2022, likely because the

relatively dry soil conditions in September 2022 increased the IWG's reliance on AM fungi for accessing water and nutrients, as shown by Oliveira et al. (2022), who stated that water stress increases the mycorrhizal colonization of a drought-sensitive soybean cultivar in a greenhouse after 3 and 7 days of inoculation. Many studies have also provided evidence that AM fungi alleviate drought and nutrient stress on plant growth (Begum et al., 2019; Gao et al., 2022; Marro et al., 2022). On the other hand, annual wheat crops had less dominant AM fungi, probably due to their very intense management compared to perennial crops, particularly in terms of the higher level of inorganic nitrogen fertilizer used, the frequency of tillage, and the use of fungicides. Moreover, annual wheat shoot was harvested in this study approximately two weeks before the sampling in September. We assume that the plant and mycorrhizal symbiosis in the annual wheat crops was weaker in September due to the lack of any photosynthesis carbon input from plant shoots.

The G^- bacteria:G^+ bacteria ratio, which indicates the relative carbon availability for soil bacterial communities in organic soils (Fanin et al., 2019), was also influenced by the cropping systems. The proportion of G^- bacteria relative to G^+ bacteria in perennial IWG-alfalfa intercropping was higher than that of IWG sole cropping in September 2022, indicating that higher amounts of labile carbon exist in the intercropping system that contains legume alfalfa. This is likely because alfalfa provided labile carbon from root exudates, which favours the G^- bacteria since they are more dependent on simple carbon compounds that are relatively labile and derived from plants, while G^+ bacteria are more dependent on complex carbon compounds derived from soil organic matter that are more recalcitrant (Kramer and Gleixner, 2008; Fanin et al., 2019). The biennial ley crop containing alfalfa and other legumes like white clover and red clover also had a higher G^- bacteria: G^+ bacteria ratio than the IWG sole crop at 15–40 cm in September 2022, likely because the alfalfa together with other legumes in ley cropping increased the rhizosphere volume. The rhizosphere usually harbours more G^- bacteria such as nitrogen-fixing rhizobia and fewer G^+ bacteria (Liang et al., 2011). Furthermore, the increased plant diversity in a plant mixture increases soil moisture, thus promoting the relative abundance of G^- bacteria (Chen et al., 2019).

According to this study, perennial cropping systems significantly increased the total microbial biomass mainly in the upper soil layers (0–30 cm) compared to conventional annual wheat cropping systems, which was contrary to the results reported by Taylor et al. (2024), who found that in deeper (30–60 cm) soil layers, IWG had a higher total microbial biomass than annual wheat. They also found that in the upper (0–15 cm and 15–30 cm) soil layers, IWG had a similar total microbial biomass compared to annual wheat. The higher total microbial biomass in IWG occurring in the upper soil layers in our study was probably due to the distribution of the IWG's roots. As observed during soil sampling for our study and in line with an earlier study by Audu et al. (2022), we found that the IWG roots in our field were more densely distributed in the shallow soil layer (0–5 cm) than in the deeper soil layer (30–40 cm). This also in agreement with a recent study published by Rakkar et al. (2023) showing that an IWG sole crop and IWG-alfalfa intercrops have their highest root biomass in the 0–15 cm soil layer, and the root biomass of the IWG sole crop decreased dramatically in the 15–30 cm, 30–40 cm and 45–60 cm soil layers at the Lamberton and Rosemount research centres in the US in 2018 and 2019. The local soil texture, fertility, pH and climate play important roles in determining IWG root biomass distribution and the biomass of microbial groups. We believe that IWG roots were more densely distributed in the upper soil layers in our research field due to the specific sandy loam soil type and the humid local climate, both of which contributed to the higher total microbial biomass in the upper soil layers. However, the fact that a higher bacterial growth rate was observed at almost all soil depths in the perennial IWG sole cropping compared to annual wheat cropping indicates that bacteria were more active and had a faster biomass turnover at all soil depths in the perennial cropping, likely due to the increased root exudates and soil organic matter input during the IWG growing seasons. The faster turnover also increased the total bacterial biomass and likely the necromass as well, which translated into the observed increase in soil total carbon concentration.

4.3. Higher crop perenniality reveals greater total carbon accumulation

For a given carbon concentration and soil thickness, the quantity of soil carbon per unit area depends on soil bulk density, which also varies with management, soil depth and other properties. In our study, if the soil bulk density were higher in perennial IWG cropping than annual cropping due to compaction from lack of tillage, then perennial crops would have a denser soil mass sampled than the annual crop at the same soil depth. Even with a potentially denser soil mass in the perennial crops, the carbon concentration was higher than that in the annual crop plots (where lower bulk density can be assumed), indicating that soil carbon in perennial cropping would been even higher if soil samples were taken and compared at an equivalent soil mass, and our current soil carbon estimates might underestimate the amount of carbon present in the perennial cropping system. Although our carbon estimates might not be ideal, our main finding that perennial crops hold more carbon would not be changed if soil bulk density measurement were applied in the field.

Perennial crops and organic wheat in this study have been applied with organic fertilizer every year. The higher soil carbon in perennial cropping could be partly from the organic fertilizers. However we found that the yearly carbon input from organic fertilizers was only 1.05%–2.81% of soil total carbon at 0–5 cm in perennial cropping, depending on fertilizers' type and amount. The carbon input from organic fertilizer for annual wheat in 2022 was 1.24 %. This 1.24% extra carbon input from organic fertilizer did not significantly influence the soil total carbon accumulation of annual wheat, if we compare the soil carbon concentration and mass between organic annual wheat with conventional annual (that applied only with inorganic fertilizers). In perennial cropping systems, if all carbon from organic fertilizers remained in the soil from 2017 to 2022 and was not respired away by soil microbes, the total carbon input from organic fertilizers to soil total carbon at 0–5 cm would be around maximum 10.6%. Therefore, the higher soil carbon accumulation in perennial cropping than conventional annual wheat were more likely a result of perennial crop itself and management such as no tillage rather than organic fertilizer application. We see a positive correlation between crop perenniality and soil total carbon concentration. Perennial crops continuously provide organic substrates via root exudates and residues for microbes the whole year round, which provides energy for soil microbes and carbon for biosynthesis (Sun et al., 2016), while annual crops provide substrates for four to five months per year. We believe that a larger amount of crop residues and root exudates in perennial cropping systems increases soil microbial biomass and microbial biomass carbon, and thus soil total carbon accumulation. The soil microbial biomass carbon positively correlated with soil total carbon concentration, and perennial cropping systems have higher microbial biomass than annual cropping systems at 0–30 cm, indicating that perennial crops have the potential to increase soil carbon sequestration in shallow soil layers. The greater soil carbon accumulation in perennial cropping systems determined in this study is partly attributable to the fungal-based food webs which have been shown to retain greater ecosystem nitrogen and carbon due to the slower rates of nitrogen cycling (Wardle et al., 2004; de Vries and Bardgett, 2012) and carbon turnover (Holland and Coleman, 1987; Bailey et al., 2002; Six et al., 2006) than bacteria-based food webs. With IWG, the greater soil carbon concentration in shallow soil layers aligns with the results reported by Sprunger et al. (2019), who found that after 4 years, IWG had significantly larger amounts of labile soil carbon and root biomass relative to annual wheat at 0–10 cm. Furthermore, at the deeper soil layer of 30–60 cm, another study (Audu et al., 2022) showed that IWG increased soil organic carbon and microbial biomass and activities compared to

organic and conventional annual crops. Our study confirmed that fungal-abundant agricultural soils under perennial cropping sequestered more carbon than bacteria-abundant soils, indicating that soil management through cultivating perennial crops can increase microbial biomass carbon and thus sequestrate more carbon in agricultural soil, although the stabilization of this soil carbon can be influenced by other abiotic and biotic factors which need to be studied further in the future.

5. Conclusion

We conclude that the integration of perennial cereal crops into agroecosystems traditionally dominated by annual crops will enhance soil quality in terms of increased soil microbial biomass, bacterial activity, microbial biomass carbon, and soil total carbon concentration in the upper soil layers. Such changes are likely to improve the quality and fertility of the soil, in favour of quality and quantity of crop production. In addition, it is likely that subsequent reduced tillage activities will facilitate the downward translocation of the soil carbon into the deeper soil profile. Such translocation could potentially support the desired carbon sink function of agricultural soils, as a climate change mitigation strategy. Although climatic conditions are the main drivers of soil microbial communities, our study reveals that at the management level, the cultivation of crops with high perenniality will shape the soil microbial structure towards a more fungal abundant community with higher microbial activity. However, the characteristics of the different fractions of the increased soil total carbon, including the contribution of perennial crop root exudates, as well as the interaction with the soil microbial community and the soil matrix, need to be further researched to better understand the dynamics between the nutrient supply by decomposition and the carbon sequestration capacity, provided by perennial crops. Such understanding is a prerequisite for determining future implementation strategies and science-based practical advice to farmers.

CRediT authorship contribution statement

Shoujiao Li: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ana Barreiro:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Juan Pablo Almeida:** Writing – review & editing, Software, Resources, Data curation. **Thomas Prade:** Writing – review & editing, Supervision. **Linda-Maria Dimitrova Mårtensson:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2024.109621.

Data availability

I have shared the link to my data at the Attach File step.

Data for "Perennial crops shape the soil microbial community and increase the soil carbon in the upper soil layer" (Original data) (Mendeley Data)

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