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Introducing Perennial Grain in Grain Crops Rotation: The Role of Rooting Pattern in Soil Quality Management

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Abstract: The use of the perennial grain intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) may have the potential to sustain soil health and fertility through the development of an extensive root system. However, references are scarce to demonstrate its potential influence in a context of a limited perennial grain growth phase, integrated into annual grain crops succession. This study aims at determining how early a perennial crop rooting system differs from that of an annual crop through root development and root traits and microbial indicators. Our results indicate that the two-year-old intermediate wheatgrass promotes a denser and deeper rooting system with proportionally more root biomass and length deeper in the soil profile. From the first growing season, the perennial grain demonstrated a suite of root traits typical of a more resource-conservative strategy, and more belowground-oriented resource allocation. Soil fungal biomass indicators were enhanced. Arbuscular mycorrhizal fungi (AMF) indicators were notably found to be improved at 1 m depth during the second growing season. This study provides evidence that grain-based agriculture can benefit from the potential of deeper and long-lived root systems of intermediate wheatgrass to manage soils. The periodic use of a short-term perennial phase in the crop rotation has the potential to improve soil functioning in the long term.

Keywords: perennial grains; rooting system; root traits; soil microbial indicators; soil quality; arbuscular mycorrhiza; crops rotation

1. Introduction

The recent development of perennial grain breeding programs has highlighted the value of the wheat wild-relative 'intermediate wheatgrass' [1] (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) to support the transition to multifunctional agroecological systems [2,3]. In addition to its ability to produce an interesting forage-grain dual income [4,5], its regrowth capacity for several years would achieve substantial production at minimal soil and environmental costs [6]. In contrast to the recurrent use of annual crops, the use of intermediate wheatgrass has been suggested to sustain soil fertility through the development of an extensive root system beneficial to a range of soil functions [6–8]. The extended lifetime of perennial grains improves the capacity to access soil resources through higher colonization of deep soil horizons [9–11] and increased resource allocation towards belowground plant growth [12]. The use of perennial crops is likely to have a range of

effects on ecosystem processes and the provision of ecosystem services due to the large differences in plant traits between annuals and perennials [13–15]. These includes the promotion of the soil organic carbon pool [13,16,17], better retention of nutrients [18], higher water storage capacity and uptakes [11], improved soil stabilization and aggregation [19,20], lower soil disturbance, and a shift of soil microbial communities [13,19,21,22].

Greater investment in belowground biomass with intermediate wheatgrass has been demonstrated several times [12,23], including greater root carbon and nitrogen content, and was associated with benefits observed on leaching reduction and enhanced soil microbiota [9,22,24,25]. In spite of the increasing literature on perennial grains and intermediate wheatgrass, studies have not thoroughly investigated issues associated with rooting patterns. Data on intermediate wheatgrass often involve aging stands (>two years old) rather than new plantings (<two years old). Compared to long-term grasslands [13,19], the integration of a perennial grain into grain crop rotation may be potentially implemented over a short timeframe (2 to 3 years as a maximum). This would likely lead to a smaller impact on soil processes and properties [26]. Therefore, it remains uncertain if the short-term use of a perennial grain can effectively enable increased belowground productivity, influence soil microbiology, or allow the establishment of root-microbe symbiosis, which are needed to confer the benefits of intermediate wheatgrass on the soil. The value of integrating a perennial phase within a grain crop rotation is then strongly dependent on the rapidity of rooting system development and its capacity to sustain soil services.

The amount and timing of these benefits are currently the cornerstone to ensure desirable and profitable use of intermediate wheatgrass, especially as grain yields are much lower compared to annual counterparts and might counteract the potential benefits to the soil [5,9,27]. The dynamic of perennial rooting systems in cropping systems is therefore critical to designing the 'safe operating space' [26] that takes advantage of a maximum services while limiting drawbacks from disservices (e.g., grain yields penalties).

In studying rooting systems, morphological root traits (e.g., specific length, length density, tissue density, diameter, vertical distribution) are used and recognized as good indicators of plant–soil processes (e.g., exudation, water and nutrients uptakes, tissue decomposability) and are useful in discussing the ecosystem services likely to be provided by plant communities (e.g., soil aggregation, water retention, carbon storage) (Figure 1) [28–32]. Complementarily, soil microbial indicators have been used to inform about soil–root interactions. Microbial biomass, community structure and catabolic diversity are particularly used to investigate carbon and nitrogen cycles and used as proxies for inputs of litter and root-derived compounds to soil [33–37]. Mycorrhizal fungi are beneficial root symbionts that participate in root system functioning and impact the soil through its hyphal network [38–40].

Here, we investigated root system development and changes in root traits of young stands of intermediate wheatgrass, from establishment to the next cropping season, comparing them to a continual annual grains crop rotation, to assess the potential to improve soil components within a limited period after establishment. We determined how rooting pattern, early root development, and a range of root traits differ in perennial and annual crops, in the topsoil to deeper soil layers. We further tested the impact of root systems on soil microbial communities, including arbuscular mycorrhiza, through the evaluation of microbial indicators. More specifically, we hypothesized that intermediate wheatgrass would demonstrate, first, a denser and deeper rooting pattern as compared to annual grains, indicating higher belowground investments. Additionally, under young perennial plants there would already be an increase in microbial organism groups, indicating an improvement in soil functioning.

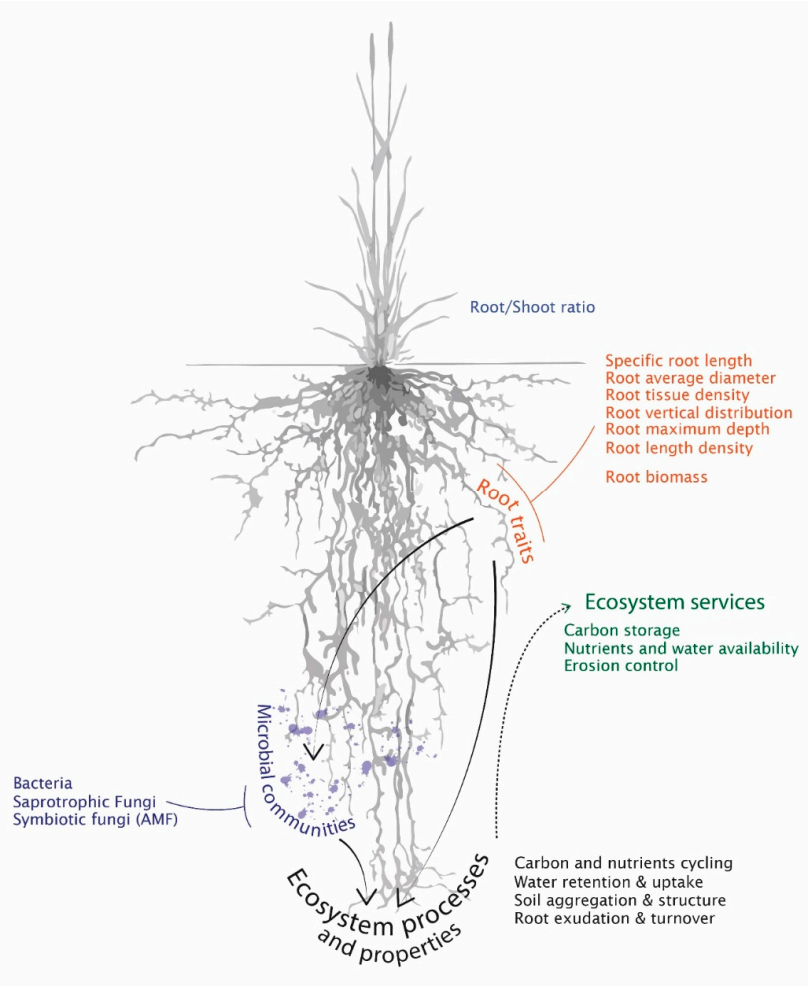

Figure 1. Influence of root traits and microbial indicators on soil ecosystem processes.

2. Materials and Methods

2.1. Site, Soil and Sampling Description

The experiment was been conducted on a no-tilled field, managed under direct sowing and using a three-year rotation of annual grains, rapeseed-winter wheat–winter rye/barley. The experimental site was located at Maubec (50°37′ N, 4°48′ E), in the South East of France. The soil was a brown luvisol [41], with main properties including a sandy-loam texture, 2.4% of organic matter, C:N ratio of 9.0 and a pH of 6.7 in the 0–30cm layer, with no significant differences among the plots used for comparing the annual and perennial treatments (see Table 1). The first and second years of our trial were characterized by 733 and 746 mm of annual precipitation, and 12 and 13 °C annual average daily temperature, respectively. Looking at the spring periods, the first and second years were characterized by 179 and 92 mm of rainfall, respectively, and 17.9 and 16 °C average daily temperature, respectively. To allow real-size management practices and farming purposes over years, and based on the homogenous soil characteristics (Table 1), the field was divided into two halves (along a north–south axis), one to grow the perennial grain 'intermediate wheatgrass Kernza©' (seed material provided by The Land Institute, a non-profit organization, Salina, Kansas, USA) and the other to maintain the regular annual grain rotation. The experiment was established in fall 2017 and studied over two consecutive years. After the rapeseed harvest, intermediate wheatgrass was sown on the perennial treatment and winter wheat (*Triticum aestivum*), followed by winter rye (*Secale cereale*) the second year, for the annual treatment. One post-emergence broadleaf herbicide application was used at each annual and perennial crop establishment to minimize weed development. Data were collected through 6 replicated sampling

zones equally spread along an east–west transect on each crop treatment, which was identified as the only natural field gradient due to a slight slope (~3%) and water drainage direction. All samples were collected along this 90m east–west sampling transect and 20 m away from the perennial–annual treatment boundary. Six pairs (row + inter-row) of soil cores were taken at the end of the first spring (6 June 2018), the regrowth period (20 November 2018) and the second spring (13 June 2019). Four soil profiles were dug for each crop treatment at the end of the second spring (14 June 2019) along the sampling transect. Soil cores and soil profiles were obtained in June to avoid unfavourable sampling in dry conditions later in summer, and as root systems growth is greatly reduced after flowering, especially for annuals which are harvested in early July. Each soil core (8 cm of diameter) to 100 cm depth was divided into four sections to distinguish between the 0–10, 10–30, 30–60, and 60–100 cm soil layers. All soil cores were analysed for root biomass, root traits, and microbial indicators as described below. All soil profiles were dug down to 160 cm depth and include two additional soil layers, 100–130 and 130–160 cm, to analyse root colonization and distribution over a greater depth. Soil samples dedicated to root measurements were stored at −20 °C until further processing, whereas samples dedicated to microbial analysis were stored at 4 °C and processed within 24 h.

2.2. Root Traits

2.2.1. Root Biomass

Root biomass was measured on each replicated soil cores, for 0–10, 10–30, 30–60 and 60–100 cm depths. After a brief soaking (15 min) where soil samples were placed in buckets filled with water, soil was washed off the roots under running water over a set of two sieves of 1 and 0.2 mm mesh-size. After the soil had been removed, all roots (only roots of the first to third order were present) were collected from sieves manually and considered as fine roots. Roots were then transferred and spread in a flat tray filled with water in order to remove any remaining impurities. Roots were not sorted between live and dead roots. Roots were oven-dried for 48 h at 60 °C and weighed. Finally, the root biomass of each soil sample was determined by averaging the root mass of the row and inter-row samples.

2.2.2. Specific Root Length, Average Root Diameter, Root Tissue Density, and Root Length Density

Sub-samples of roots were taken after washing for the root biomass described above for the purpose of root morphology measurements. Roots where spread in a flat transparent tray to avoid any roots overlapping, digitized at 600 dpi with a flatbed scanner (Epson V800 Pro) and analysed using a digital image analysis system (WinRhizo, version 2019; Regent Instrument) to obtain root length, root diameter and root volume. Root volume measurements were calculated from the sum of root volumes per diameter class (by 0.05 mm steps). Root dry mass was obtained after oven-drying scanned roots for 48 h at 60 °C. From these estimates, we calculated the average root diameter (mm), specific root length (m g^{-1}) and root tissue density (g cm^{-3}). Root length density (cm cm^{-3}) was obtained by multiplying root biomass in the soil core section by its estimated specific root length and dividing it by the volume of the soil core section.

2.2.3. Maximum Rooting Depth, % of Soil Colonized by Roots, % of Total Root Observations

In each soil profile replicate, a 70 cm wide and 160 cm deep grid of 2 × 2 cm squares was placed and fixed along the soil profile with the top of the grid at the soil surface level. Each 2 × 2 cm square of the grid was observed and defined by the presence or absence of roots, generating a two-dimensional map of the root system along the soil profile. The number of grid cells where at least one root was observed was recorded for each of the 0–10, 10–30, 30–60, 60–100, 100–130, 130–160 cm soil layers. The maps were used to analyse differences in vertical root distribution. We calculated both the percentage of grid cells with roots within each soil layer and the percentage represented by each soil layer in the total number of grid cells observations with roots (from 0 to 160 cm). Finally, the maximum rooting depth was determined as the maximum depth at which roots were observed.

Table 1. Baseline soil profile characteristics (numbers in brackets indicate standard errors). Both perennial and annual crops areas are characterized through 6 different soil samples, and compared by means comparison (Student tests). *p*-value are indicated as follow: NS = no significant (*p*-value > 0.05), S = significant (*p*-value < 0.05).

Soil Depth Layer (cm)	SOIL Texture	Crop Treatment	Organic Matter	Total Nitrogen	Phosphorus	C:N Ratio	pH	Biological Activity Traces	Macro-Porosity
			(g kg^{-1})						
0–30	Sandy loam	Annual	23.8 (1.5)	1.4 (0.5)	0.04 (0.006)	8.3 (0.3)	6.7 (0.04)	Strong	High
		Perennial	24.6 (0.9) NS	1.9 (0.4) NS	0.03 (0.01) NS	9.7 (0.4) NS	6.8 (0.04) NS		
30–60	Sandy clay loam	Annual	9.7 (0.9)	0.7 (0.1)	0.01 (0.003)	8.4 (0.05)	6.7 (0.02)	Strong	High
		Perennial	9.9 (0.6) NS	0.6 (0.2) NS	0.01 (0.002) NS	8.2 (0.08) NS	7.0 (0.04) NS		
60–100	Silty clay loam	Annual	5.7 (0.6)	0.4 (0.08)	0.009 (0.002)	6.0 (0.09)	7.2 (0.03)	Moderate	Moderate
		Perennial	6.0 (0.7) NS	0.4 (0.1) NS	0.01 (0.004) NS	5.4 (0.04) NS	6.8 (0.02) NS		
100–160	Clay loam		-	-	-	-	-	Low	Low–temporary hydromorphy

2.3. Aboveground Biomass

Aboveground biomass was sampled on 18 June 2018 and 20 June 2019. These times corresponded to the grain filing period of the annual wheat and rye, respectively, in 2018 and 2019, whereas it represented the flowering period of intermediate wheatgrass. An additional aboveground biomass sampling was performed on 21 November 2018 for the perennial grain treatment, corresponding to the end of its post-harvest regrowth period. Aboveground biomass was sampled in each treatment close to each soil core pair. Dry matter (DM) was determined by taking samples by cutting all plant tissue 5 cm above the soil surface in a 0.5 m^2 quadrat, drying at 80 °C for 48 h and then weighing. Root to shoot ratio was then determined by scaling root biomass and aerial biomass per unit ground area sampled and dividing root biomass by shoot biomass.

2.4. Soil Microbial Analysis

Phospholipid and neutral lipid fatty acids (PLFAs and NLFAs) are cell-wall compounds, which can be used to study microbial communities and estimate microbial biomass. Fatty acids (referred hereafter as "markers") were extracted from the 0–10, 10–30, 30–60, and 60–100 cm soil layers using the protocol of Frostegård and Bååth [42] using CHCl3:MeOH:citrate buffer (1:2:0.8 v/v/v) and detected by gas chromatography with a flame ionization detector (GC-17A, Shimadzu). The sum of the markers considered to be predominantly of bacterial origin (i14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:1ω8, i17:0, a17.0, 17:0, cy17:0, 18:1ω7 and cy19:0) was used as an index of the bacterial biomass. The sum of the markers 18:1ω9, 18:2ω6 and 16:1ω5 was used to calculate total fungal biomass, where the marker 18:1ω9 is a general fungal indicator, 18:2ω6 is indicative for saprotrophic fungi [42–44], and 16:1ω5 for arbuscular mycorrhizal fungi (AMF) [45]. The marker N-16:1ω5 (neutral lipid) is used to control for the validity of the marker P-16:1ω5 (phospholipid) as an AMF indicator, since the ratio between the two is high in AMF [46] and because N-16:1ω5 is highly correlated with AMF spore counts [47]. Therefore, the final ratio between the markers N-16:1ω5 and P-16:1ω5 is used an overall indicator of AMF structure abundance [48].

2.5. Statistical Analysis

Consistently with the 6 replicated sampling zones, each analysis was performed on 12 measures (6 annuals and 6 perennials) for each sampling period, with the exception of (i) the post-harvest regrowth (fall) period that only concerns the perennial treatment, and (ii) measures of root colonization (Section 3.2) which had only four replications per treatment due to the difficulty to perform soil profiles. Statistical analysis was conducted in R [49]. All above and belowground variables were analysed for differences between perennial and annual grain treatments using mixed models (lmerTest package) [50]. Crop treatment, soil depth and their respective interactions were treated as fixed effects. The absence of a randomized split-block design due to farming constraints could then imply that a crop treatment effect might also reveal a location effect. However, the initial field design was carefully chosen to ensure soil homogeneity, and initial soil profiles were performed to confirm similar soil characteristics, depth and nature of deep soil layers (Table 1). The amount of sampling points (6 replicates per treatments along the transect at each sampling period) was defined to take account of potential variability. The sampling period, sampling replicates and interactions with the other factors were treated as random effects. The covariance structure assigned in the repeated measures statement was compound symmetry. When crop treatment effect was found to be significant (p-value < 0.05), means were compared between annual and perennial treatment with an adjusted Tukey's pairwise mean comparison (post hoc analysis, $\alpha = 0.05$). Analyses of the % of soil colonized by roots, % of total root observations and the maximal rooting depth did not include a sampling period factor due to the unique data collection period performed at the second spring growing season. All graphics were produced with ggplot2 package.

3. Results

3.1. Above and Belowground Crops Biomass

Higher root biomass was observed under the perennial grain compared to annual wheat at 0–10 cm depth in June 2018 (first spring) (Figure 2). Perennial root expansion was observed during the regrowth period, with higher total root biomass compared to the values observed during first spring. Overall, between the end of the first and second spring growing seasons, perennial above and belowground biomass increased by 52% and 111%, respectively. Consequently, root biomass was higher under the perennial treatment in each soil layer, leading to more than three-fold higher total root biomass (5.3 tonnes more of dry roots hectare^{-1}) compared to annual rye at the same time. Aboveground biomass of the perennial grain was also 39.4% higher compared to annual rye. The perennial root biomass at the second spring was also significantly higher as compared to the overall root biomass production of annual grains (annual wheat roots + annual rye roots) during the two growing seasons (p-value < 0.001). Looking at the root to shoot ratio, perennial roots represented 38.6% of the aerial biomass produced at the first spring growing season, against 24.4% for the annual wheat at the same time, while increasing to 55.3% at second spring time (June 2019), against 22.9% for the annual rye. This greater belowground allocation of the perennial coupled with lower grain yields led to a lower harvest index (p-value < 0.001, data not shown). Grain yields for the perennial and annual treatment were 1.2 and 7.8 t/ha in the first year, and 1.3 against 8.1 t/ha in the second year, respectively.

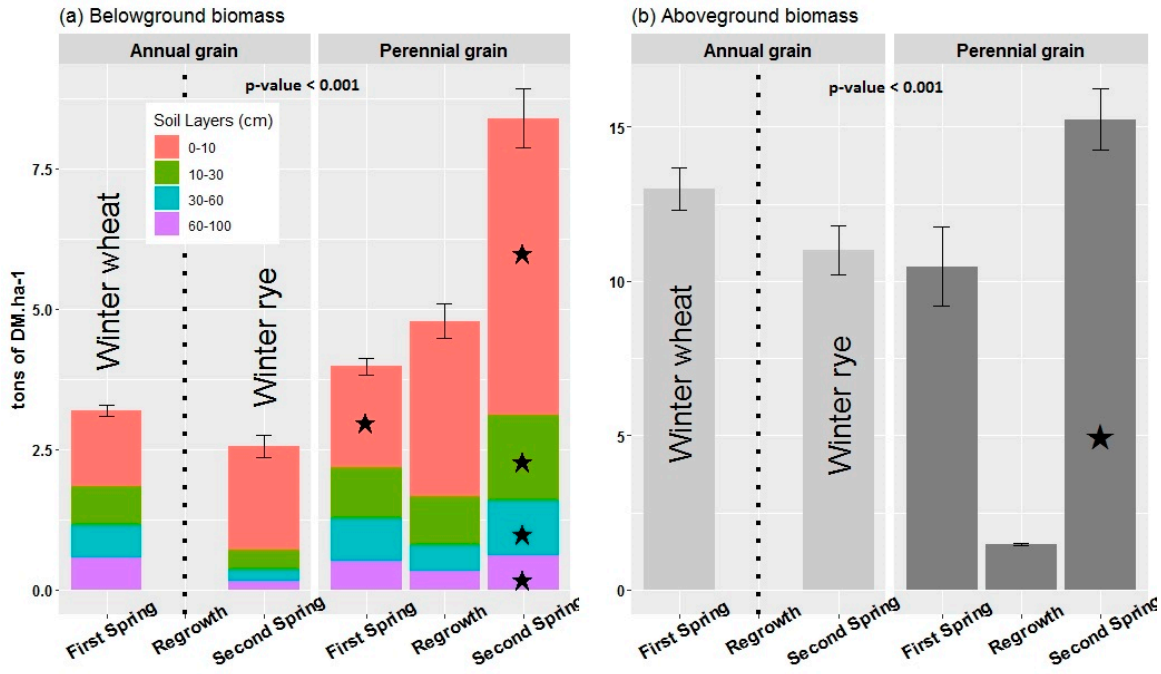

Figure 2. Mean root dry biomass (**a**) and aboveground dry biomass (**b**) measured under annual and perennials grains during two crop seasons. Statistical differences (post hoc analyses) between annual and perennial grains are indicated by ★. Error bars indicate standard errors, from 0 to 100 cm depth.

3.2. Root Colonization of the Soil Profile

The % of soil colonized by roots (Figure 3a) and % of total root in each soil depth (Figure 3b) observed in the second spring growing season both indicated a significant effect of crop treatment. Except for the topsoil layer (0–10 cm), all soil layers had significantly higher colonization under perennial grain cultivation. The perennial root system showed a thorough soil colonization until 60cm, with 96 and 83% of colonized soil for the 10–30 and 30–60 cm soil layers, respectively, whereas the annual root system showed only 62 and 54% of soil colonized soil for the same layers. Similarly, for the

deeper soil layers of 60–100, 100–130 and 130–160 cm, the perennial root system colonized as much as 66, 58 and 54% of the observed soil profile, respectively, whereas the annual root system showed relatively low soil colonization of 35, 27 and 14%, respectively, at these depths. The maximum rooting depth of annual grain (141.5 cm ± 6.8 SE) was less than that of the perennial grain, where the maximum rooting depth was >160cm in each soil profile replicate. The root vertical distribution indicates that the perennial root system showed a more even distribution as compared to the annual root system (Figure 3b). The annual root system had a stronger preferential allocation of annual roots in upper soil layers (on average, 68% of the annual root system is found between 0 and 60 cm, while it is only 47% for the perennial root system). In contrast, as much as 30% of the perennial root system was found in the deeper soil layers (100–160 cm), as compared to only 16% for the annual grains.

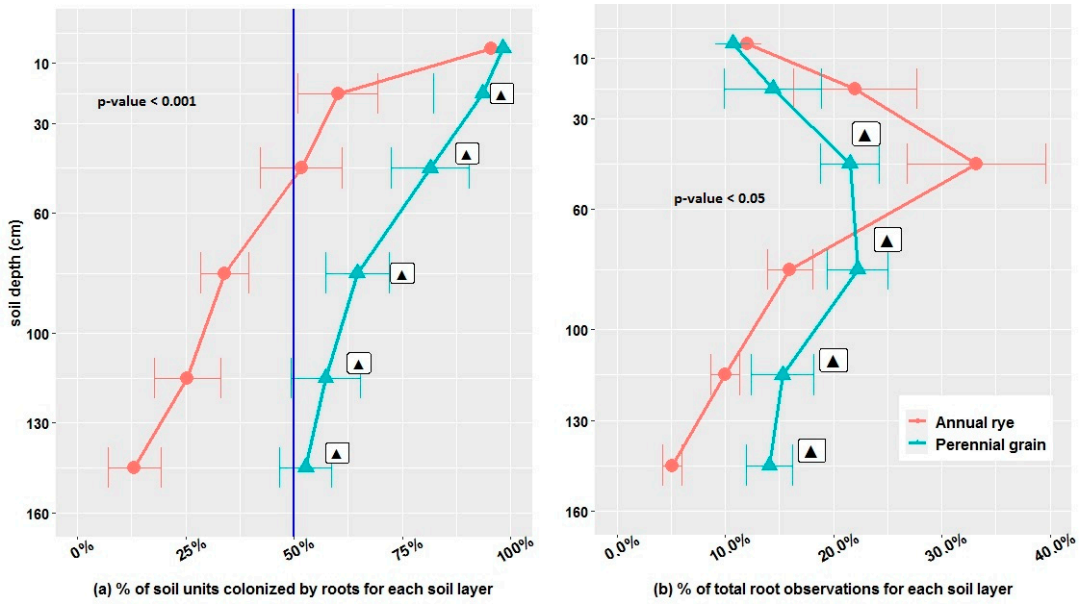

Figure 3. Mean root colonization of the soil profile observed at the second crop season, under intermediate wheatgrass and annual winter rye. Standard errors are represented by error bars. Statistical differences (post hoc analyses) between annual and perennial grains are indicated by ▲.

3.3. Specific Root Length, Diameter, Tissue Density, and Length Density

Specific root length (SRL), mean root diameter (RD) and root tissue density (RTD) all demonstrate the different rooting patterns between the annual and perennial grains, where annuals have higher SRL (Figure 4a) but a lower RD and RTD (Figure 4b,c). In general, SRL increased and RD and RTD decreased with soil depth. In comparison to SRL, RD and RTD, the root length density (RLD) is associated to a given volume of soil. Significant differences between annual and perennial grains were mainly observed in the second year (Figure 4d), as the higher RLD under the perennial grain (from 10 to 100 cm) was associated with increased root biomass (Figure 2). RLD was higher for each crop in the 0–10 cm soil layer due to the highest concentration of roots in the top layer (Figure 2). Differences between annual and perennial grains through volume-based root measures (root biomass, RLD) were associated with perennial growth duration (establishment–regrowth), while single root traits are associated with annual–perennial crop type, independent of growth duration.

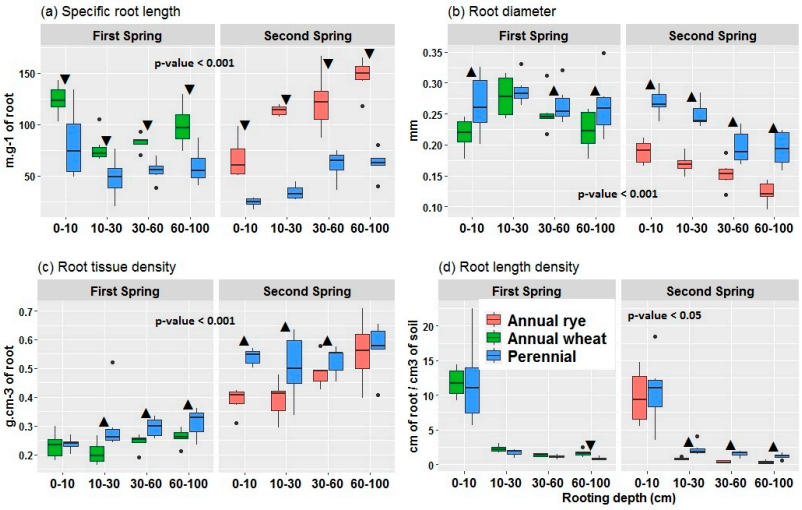

Figure 4. Morphological root traits under annual wheat and intermediate wheatgrass, and between annual rye and the perennial during the first and second growing seasons. *p*-values refer to the difference between perennial and annual crop treatment. Statistically significant differences (post hoc analyses) are illustrated by ▲ (higher values for the perennial grain) or ▼ (lower value for the perennial grain).

3.4. Soil Microbial PLFA and NLFA Markers

Bacterial markers did not respond to the crop treatment (Figure 5a), while both the overall fungal markers (Figure 5b) and AMF markers did (Figure 5c,d). Fungal markers, including AMF, at 0–10 cm depth were higher under the perennial treatment in both first and second spring samplings compared to the annual crops, winter wheat and winter rye, respectively (Figure 5b–d). The abundance of AMF N-marker was higher for each soil layer from 0 to 100 cm depth in the second spring, showing five times greater abundance compared to the annual winter rye. The ratio between the two AMF markers (N-to P-marker) was significantly higher than the annual crop in all soil layers under intermediate wheatgrass in the second experimental year and in the deepest soil layer (60–100 cm) in the first experimental year (Figure 6). The ratio between the two markers generally increased with soil depth (p-value < 0.001).

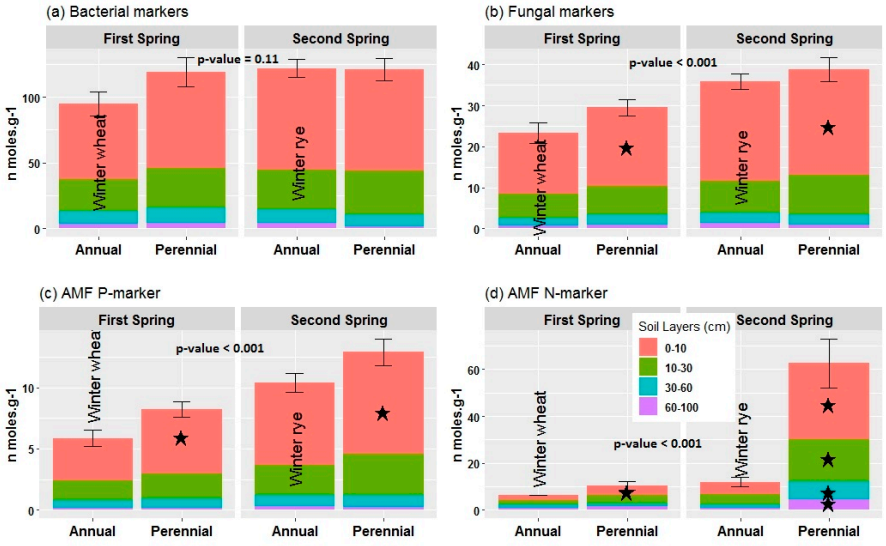

Figure 5. The PLFA and NLFA markers in annual wheat and intermediate wheatgrass, and between annual rye and the intermediate wheatgrass during the first and second growing seasons. Statistical differences (post hoc analyses) between annual and perennial grains are indicated by ★. Error bars indicate standard errors, from 0 to 100 cm depth.

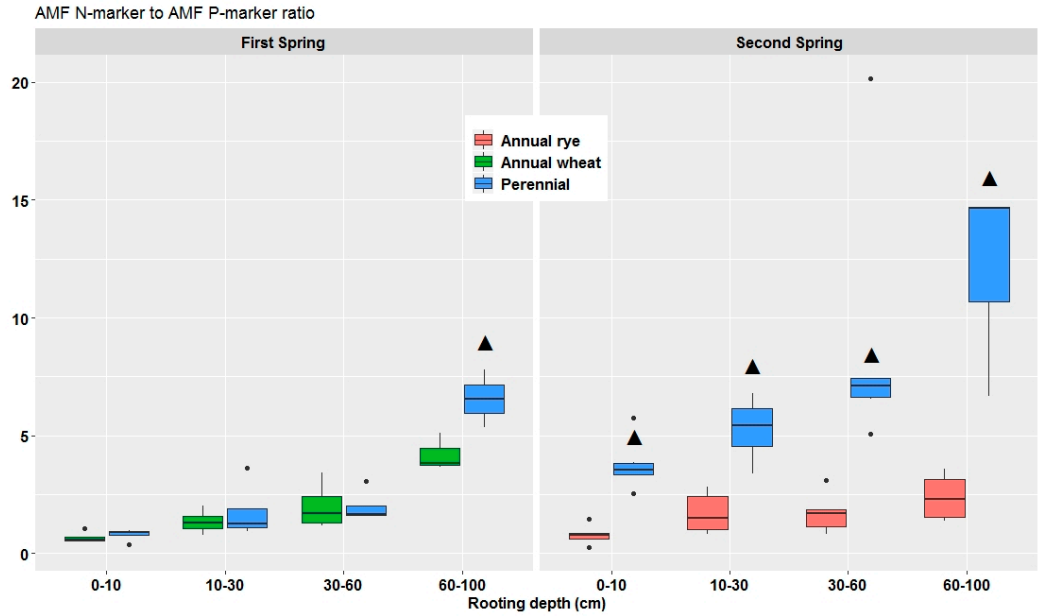

Figure 6. NLFA to PLFA marker ratio indicating (AMF). Statistical differences (post hoc analyses) are illustrated by ▲.

4. Discussion

4.1. Rapid Rooting and Microbial Pattern Changes

Compared to annual crops, and within two years after establishment, stands of intermediate wheatgrass showed a denser and deeper rooting system profile. This highlights how perennial grains allocate more assimilates to belowground parts. Total root biomass produced by intermediate wheatgrass after two years was higher than the sum of the root biomass produced by both annual crops during the two growing seasons. Furthermore, the dense and deep root system observed with intermediate wheatgrass demonstrates the ability of the perennial grain to explore the entire soil profile and subsoil layers, permitting access to greater amounts of soil resources and suggesting a potential impact of its root functioning on a larger volume of soil. Intermediate wheatgrass was able to produce higher amounts of root biomass in the topsoil layer by the first spring, and then in the entire soil profile during the following growing season including the fall period, as compared to the annual grains succession.

Another important aspect of perennial grain rooting pattern lies in the higher average root diameter and tissue density. These traits are generally related to a more resource-conservative strategy [51,52] and suggest enhanced root storage functions and inputs of more recalcitrant root tissue in soil [32]. These functional 'conservative' traits have been described in grasslands as traits favouring slower recycling, higher nutrients residence time in tissues, and promoting ecosystem nutrient cycles closure, rather than leaks outside the system [53]. The lower specific root length of intermediate wheatgrass reflects two additional trends. First, this lower SRL coincides with a higher root to shoot ratio, suggesting that the higher cost of soil exploration per unit root biomass is at least partly compensated by higher relative investment of perennial grains towards the root system [54]. Second, lower specific root length, resulting in lower competitive ability for resource acquisition per unit of root, may generally reflect a tendency of perennial grains to rely more on mycorrhizal symbionts for their soil resource acquisition [51,55]. This pattern is indeed confirmed by our finding that AMF biomass in the soil increases under perennial grains, as discussed below.

The hypothesis of increased abundance of soil microbial communities under intermediate wheatgrass is partially fulfilled. While no significant impact of intermediate wheatgrass was observed on bacterial markers, fungal abundance seems to slightly but significantly increase rapidly in the

topsoil layers. This promotion of fungal communities likely stems from a change in root litter and organic matter quality in the soil [56–58], reflecting more recalcitrant compounds, in comparison to more readily decomposable substrates which favour bacterial growth [59]. Interestingly, the increase in AMF biomass markers under intermediate wheatgrass is indicative of the deeper change to the 1 m depth. Higher values of the AMF N-marker compared to P-marker may be linked to a substantial sporulation process [47] under the perennial grain, hypothetically linked to either a host-specific AMF growth response [60] or a greater responsiveness of perennial plants to AMF colonization [61]. Furthermore, the ratio of N- to P- AMF markers is assumed to be greatly influenced by crop mycotrophy, where higher ratios, as observed in the perennial rooting system, indicate a higher level of AMF biomass in soil [48]. Hence, evidence of improved mycorrhizal structures at depth within a short timeframe is meaningful in that it suggests further considering the importance of soil exploration and interactions under perennial grains. Increasing plant-mycorrhizal fungi associations deeper in the soil would imply that mycorrhizal associations can trade-off with the use of high specific root length [55,62] and enhance the overall root-hyphae length density in the soil. This suggests therefore an even greater zone of influence of the intermediate wheatgrass root system, where root measurements offer only a partial image of the rooting actions and processes in both the top and subsoil layers after intermediate wheatgrass establishment.

4.2. A Range of Influences on Soil Functioning and Services Supply

Extending soil cover with intermediate wheatgrass is surely the first parameter that drives soil protection and erosion control [63]. Additionally, both higher root length density and fungal biomass act to improve soil cohesion and aggregation [20,21,64], leading to multiple benefits including carbon and nitrogen retention [65–67], water infiltration and holding capacity [68], and soil stability and resistance to soil water erosion [64,69,70].

Larger root biomass means higher amounts of carbon and nitrogen allocated to the whole root system [24,25]. However, while root biomass and litter inputs are ultimately the main driver of carbon and nitrogen inputs to the soil [18,71–73], root traits and rooting depth are also critical to assess the impact of roots on carbon storage and nutrient availability in soil [74,75]. In this study, as compared to the regular root litter inputs from annual crops after each growing season, the longer lifespan and higher biomass of perennial roots would imply lower organic matter inputs available for nitrifying bacteria in the short-term [30]. In contrast, annual and fast-growing species would be more prone to rapidly increasing nitrogen mineralization via higher tissue turnover [76]. Short-term benefits from perennial roots may occur through the reduction in the denitrification process by better nitrate uptake, enabled by higher root length density and soil exploration [77]. Its contribution to soil nitrogen accumulation may turn out to be substantial in the medium term (>2–3 years) as root litter inputs should increase with time, or following crop destruction, resulting in massive root litter inputs [78]. However, prior to crop destruction, the increased soil colonization of subsoil layers can be useful to mine leached nutrients, as well as to capture groundwater in times of water shortage [9,79,80]. The greater soil exploration and water capture allowed by perennials may, however, result first in mitigating yields variability and improve resiliency rather than increasing grain yields [11,81] due to the high root-shoot ratio and the low grain harvest index.

Carbon inputs into subsoil layers may lead to more protected carbon stocks, particularly via organo-mineral associations but also, on the shorter-term, via lower decomposition rates in deeper soil layers [66,73,82,83]. Higher root tissue density of intermediate wheatgrass compared to annual counterparts and in deeper soil layers also implies a slower root decomposition rate [84] and therefore medium-term build-up of soil organic carbon [17]. In contrast, the short-term root exudation rate from live roots of intermediate wheatgrass may have been reduced as compared to annual grains, by the lower specific root length [85], its slow-growing strategy and a lower net primary productivity allocated aboveground [86]. Such lower exudation rates would limit the rhizosphere priming effect [14,87],

but would also reduce the amount of labile carbon entering the soil, with a currently unknown outcome on the balance between carbon inputs and outputs.

Among the potential benefits from a greater abundance of mycorrhiza is the ability to access nutrient pools and to enhance soil aggregation [88–90]. Overall, an increase in the abundance of fungal communities has also been observed in less disturbed and extensively managed habitats with better regulation of ecosystem processes, nutrient cycling [33,91], and carbon storage in the soil [92–94]. More generally, microorganisms are providing the first source of long-term stabilized organic matter through microbial residues produced from the decomposition and metabolization of labile carbon [95,96]. The potential increased activity of fungal communities in mineral subsoil layers due to the deep perennial root carbon inputs could be especially valuable for long-term carbon stabilization, which is, in part, facilitated by organo-mineral bonds [96].

4.3. Are Two Years Enough to Consider Soil Gains and Services Supply?

Intermediate wheatgrass root traits and the large variability of processes affected by them (Figure 1) points to two main highlights: (i) denser and deeper rooting system of intermediate wheatgrass generates multiple rapid benefits on soil protection, stability, and water and nutrient availability, and (ii) promotes carbon and nitrogen allocation belowground with higher residence time. However, no clear insight on whether soil health and fertility gains will be tangible in the short-term, as microbial changes were weak and mostly limited to the top soil layer (except for AMF indicators). Such uncertainties of root litter cycling imply either that carbon and nitrogen soil status would not benefit visibly from a short perennial phase integration into the crop succession, which may limit the use of perennial grains to specific vulnerable zones (e.g., erosion, leaching); or that perennial rooting would need repeated and regular integration into annual systems to be of significance, and involves important afterlife effects. Previous works suggest a clear potential for using a repeated perennial crop-grass phase in annual grain rotation [97], where benefits may be substantial on the subsequent yields of following crops [98] and/or visible through increases in soil carbon and nitrogen stocks [99,100]. The rooting pattern of intermediate wheatgrass would suggest this to be especially notable at depth.

The design of the study presented in this paper did not include the testing of the subsequent effects of large perennial root inputs, as soil measurements were made before the incorporation of the whole intermediate wheatgrass root system into the soil (in contrast to annual wheat). This issue still needs to be addressed over a longer-term perspective [13,78], but suggests that an overall positive effect of perennial wheatgrass on soil functioning may be stronger than measured herein.

Overall, it is important to consider that productive and sustainable fields rely more on the ability to maintain a functional regulation between storage (immobilization) and mineralization (mobilization) processes of carbon and nutrients in soils, rather than on one or another process separately. The balance between high C:N soil, often less intensively managed, and low C:N soil, intensively managed, has been seen as a fundamental trade-off between production and environmental purposes [97,101]. Fields that carry the capacity to ensure soil protection, to store carbon and to sustain nitrogen availability require a high degree of ecological engineering. Indeed, plant–soil interactions are context-dependent and are influenced by crop choice and sequence, and by management practices (e.g., balance between carbon harvested and returned). Therefore, the role of a short-term perennial grain phase in a crop rotation may be to improve the synchrony between nutrient availability and plant demand [102] through increased nutrient retention, diversified organic matter quality and the recruitment of microorganisms.

Depending on the nature and magnitude of services required, or disservices to avoid, the duration of the perennial phase must be a guiding operating tool and a focal area for further research.

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References

1. DeHaan, L.; Christians, M.; Crain, J.; Poland, J. Development and Evolution of an Intermediate Wheatgrass Domestication Program. *Sustainability* **2018**, *10*, 1499. [CrossRef]
2. Duchene, O.; Celette, F.; Ryan, M.R.; DeHaan, L.R.; Crews, T.E.; David, C. Integrating multipurpose perennial grains crops in Western European farming systems. *Agric. Ecosyst. Environ.* **2019**, *284*, 106591. [CrossRef]
3. Ryan, M.R.; Crews, T.E.; Culman, S.W.; DeHaan, L.R.; Hayes, R.C.; Jungers, J.M.; Bakker, M.G. Managing for Multifunctionality in Perennial Grain Crops. *BioScience* **2018**, *68*, 294–304. [CrossRef] [PubMed]
4. Favre, J.R.; Castiblanco, T.M.; Combs, D.K.; Wattiaux, M.; Picasso, V.D. Forage nutritive value and predicted fiber digestibility of Kernza intermediate wheatgrass in monoculture and in mixture with red clover during the first production year. *Anim. Feed. Sci. Technol.* **2019**, *258*, 114298. [CrossRef]
5. Jungers, J.M.; DeHaan, L.R.; Betts, K.J.; Sheaffer, C.C.; Wyse, D.L. Intermediate Wheatgrass Grain and Forage Yield Responses to Nitrogen Fertilization. *Agron. J.* **2017**, *109*, 462–472. [CrossRef]
6. Crews, T.E. Closing the Gap between Grasslands and Grain Agriculture. *Kans. J. Law Public Policy* **2016**, *26*, 274.
7. Crews, T.E.; Carton, W.; Olsson, L. Is the future of agriculture perennial? Imperatives and opportunities to reinvent agriculture by shifting from annual monocultures to perennial polycultures. *Glob. Sustain.* **2018**, *1*. [CrossRef]
8. DeHaan, L.R.; Ismail, B.P. Perennial Cereals Provide Ecosystem Benefits. *Cereal Foods World* **2017**, *62*, 278–281. [CrossRef]
9. Culman, S.W.; Snapp, S.S.; Ollenburger, M.; Basso, B.; DeHaan, L. Soil and Water Quality Rapidly Responds to the Perennial Grain Kernza Wheatgrass. *Agron. J.* **2013**, *105*, 735–744. [CrossRef]
10. Thorup-Kristensen, K.; Halberg, N.; Nicolaisen, M.; Olesen, J.E.; Crews, T.E.; Hinsinger, P.; Kirkegaard, J.; Pierret, A.; Dresbøll, D.B. Digging Deeper for Agricultural Resources, the Value of Deep Rooting. *Trends Plant Sci.* **2020**, *25*, 406–417. [CrossRef]
11. Vico, G.; Brunsell, N.A. Tradeoffs between water requirements and yield stability in annual vs. perennial crops. *Adv. Water Resour.* **2018**, *112*, 189–202. [CrossRef]
12. Sainju, U.M.; Allen, B.L.; Lenssen, A.W.; Ghimire, R. Root biomass, root/shoot ratio, and soil water content under perennial grasses with different nitrogen rates. *Field Crop. Res.* **2017**, *210*, 183–191. [CrossRef]
13. Dupont, S.T.; Beniston, J.; Glover, J.D.; Hodson, A.; Culman, S.W.; Lal, R.; Ferris, H. Root traits and soil properties in harvested perennial grassland, annual wheat, and never-tilled annual wheat. *Plant Soil* **2014**, *381*, 405–420. [CrossRef]
14. Henneron, L.; Cros, C.; Picon-Cochard, C.; Rahimain, V.; Fontaine, S. Plant economic strategies of grassland species control soil carbon dynamics through rhizodeposition. *J. Ecol.* **2020**, *108*, 528–545. [CrossRef]
15. Roumet, C.; Urcelay, C.; Díaz, S. Suites of root traits differ between annual and perennial species growing in the field. *New Phytol.* **2006**, *170*, 357–368. [CrossRef] [PubMed]
16. Dignac, M.-F.; Derrien, D.; Barre, P.; Barot, S.; Cécillon, L.; Chenu, C.; Chevallier, T.; Freschet, G.T.; Garnier, P.; Guenet, B.; et al. Increasing soil carbon storage: Mechanisms, effects of agricultural practices and proxies. A review. *Agron. Sustain. Dev.* **2017**, *37*, 351. [CrossRef]
17. Poirier, V.; Roumet, C.; Munson, A.D. The root of the matter: Linking root traits and soil organic matter stabilization processes. *Soil Boil. Biochem.* **2018**, *120*, 246–259. [CrossRef]
18. De Vries, F.T.; Bardgett, R.D. Plant community controls on short-term ecosystem nitrogen retention. *New Phytol.* **2016**, *210*, 861–874. [CrossRef]
19. Culman, S.W.; Dupont, S.; Glover, J.; Buckley, D.H.; Fick, G.; Ferris, H.; Crews, T. Long-term impacts of high-input annual cropping and unfertilized perennial grass production on soil properties and belowground food webs in Kansas, USA. *Agric. Ecosyst. Environ.* **2010**, *137*, 13–24. [CrossRef]
20. Stokes, A.; Atger, C.; Bengough, A.G.; Fourcaud, T.; Sidle, R.C.; Bengough, A.G. Desirable plant root traits for protecting natural and engineered slopes against landslides. *Plant Soil* **2009**, *324*, 1–30. [CrossRef]

21. Chantigny, M.H.; Angers, D.A.; Prévost, D.; Vézina, L.-P.; Chalifour, F.-P. Soil Aggregation and Fungal and Bacterial Biomass under Annual and Perennial Cropping Systems. *Soil Sci. Soc. Am. J.* **1997**, *61*, 262–267. [CrossRef]
22. McKenna, T.P.; Crews, T.E.; Kemp, L.; Sikes, B.A. Community structure of soil fungi in a novel perennial crop monoculture, annual agriculture, and native prairie reconstruction. *PLoS ONE* **2020**, *15*, e0228202. [CrossRef] [PubMed]
23. Sprunger, C.D.; Culman, S.W.; Robertson, G.P.; Snapp, S.S. How Does Nitrogen and Perenniality Influence Belowground Biomass and Nitrogen Use Efficiency in Small Grain Cereals? *Crop. Sci.* **2018**, *58*, 2110–2120. [CrossRef]
24. Sainju, U.M.; Allen, B.L.; Lenssen, A.W.; Mikha, M. Root and soil total carbon and nitrogen under bioenergy perennial grasses with various nitrogen rates. *Biomass Bioenergy* **2017**, *107*, 326–334. [CrossRef]
25. Sprunger, C.D.; Culman, S.W.; Peralta, A.L.; Dupont, S.T.; Lennon, J.T.; Snapp, S.S. Perennial grain crop roots and nitrogen management shape soil food webs and soil carbon dynamics. *Soil Boil. Biochem.* **2019**, *137*, 107573. [CrossRef]
26. Martin, G.; Durand, J.-L.; Duru, M.; Gastal, F.; Julier, B.; Litrico, I.; Louarn, G.; Médiène, S.; Moreau, D.; Valentin-Morison, M.; et al. Role of ley pastures in tomorrow's cropping systems. A review. *Agron. Sustain. Dev.* **2020**, *40*. [CrossRef]
27. Tautges, N.E.; Jungers, J.M.; DeHaan, L.; Wyse, N.L.; Sheaffer, C.C. Maintaining grain yields of the perennial cereal intermediate wheatgrass in monoculture v. bi-culture with alfalfa in the Upper Midwestern USA. *J. Agric. Sci.* **2018**, *156*, 758–773. [CrossRef]
28. Bardgett, R.D.; Mommer, L.; De Vries, F.T. Going underground: Root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* **2014**, *29*, 692–699. [CrossRef]
29. Craine, J.M.; Wedin, D.A.; Chapin, F.S.; Reich, P.B. The dependence of root system properties on root system biomass of 10 North American grassland species. *Plant Soil* **2003**, *250*, 39–47. [CrossRef]
30. Craine, J.M.; Tilman, D.; Wedin, D.; Reich, P.B.; Tjoelker, M.G.; Knops, J. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. *Funct. Ecol.* **2002**, *16*, 563–574. [CrossRef]
31. Craine, J.M.; Froehle, J.; Tilman, D.G.; Wedin, D.A.; Chapin, I. The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. *Oikos* **2001**, *93*, 274–285. [CrossRef]
32. Freschet, G.T.; Roumet, C. Sampling roots to capture plant and soil functions. *Funct. Ecol.* **2017**, *31*, 1506–1518. [CrossRef]
33. Bardgett, R.D.; McAlister, E. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. *Boil. Fertil. Soils* **1999**, *29*, 282–290. [CrossRef]
34. Degens, B. Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. *Soil Boil. Biochem.* **2000**, *32*, 189–196. [CrossRef]
35. Fontaine, S.; Mariotti, A.; Abbadie, L. The priming effect of organic matter: A question of microbial competition? *Soil Boil. Biochem.* **2003**, *35*, 837–843. [CrossRef]
36. Grigulis, K.; Lavorel, S.; Krainer, U.; Legay, N.; Baxendale, C.; Dumont, M.; Kastl, E.; Arnoldi, C.; Bardgett, R.D.; Poly, F.; et al. Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. *J. Ecol.* **2012**, *101*, 47–57. [CrossRef]
37. Van Der Heijden, M.G.A.; Bardgett, R.D.; Van Straalen, N.M. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* **2008**, *11*, 296–310. [CrossRef]
38. Averill, C.; Turner, B.L.; Finzi, A.C. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nat.* **2014**, *505*, 543–545. [CrossRef]
39. Gianinazzi, S.; Gollotte, A.; Binet, M.-N.; Van Tuinen, D.; Redecker, D.; Wipf, D. Agroecology: The key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* **2010**, *20*, 519–530. [CrossRef]
40. Phillips, R.P.; Brzostek, E.R.; Midgley, M.G. The mycorrhizal-associated nutrient economy: A new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytol.* **2013**, *199*, 41–51. [CrossRef]
41. FAO. *World Reference Base for Soil Resources 2014: International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; FAO: Rome, Italy, 2014.
42. Frostegård, A.; Bath, E. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils* **1996**, *22*, 59–65. [CrossRef]

43. Frostegard, A.; Tunlid, A.; Bååth, E. Use and misuse of PLFA measurements in soils. *Soil Boil. Biochem.* **2011**, *43*, 1621–1625. [CrossRef]
44. Zelles, L. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: A review. *Boil. Fertil. Soils* **1999**, *29*, 111–129. [CrossRef]
45. Olsson, P.A.; Bååth, E.; Jakobsen, I.; Söderström, B. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycol. Res.* **1995**, *99*, 623–629. [CrossRef]
46. Olsson, P.A.; Thingstrup, I.; Jakobsen, I. Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. *Soil Biol. Biochem.* **1999**, *9*, 1879–1887. [CrossRef]
47. Sharma, M.P.; Buyer, J.S. Comparison of biochemical and microscopic methods for quantification of arbuscular mycorrhizal fungi in soil and roots. *Appl. Soil Ecol.* **2015**, *95*, 86–89. [CrossRef]
48. Vestberg, M.; Palojärvi, A.; Pitkänen, T.; Kaipainen, S.; Puolakka, E.; Keskitalo, M. Neutral lipid fatty acid analysis is a sensitive marker for quantitative estimation of arbuscular mycorrhizal fungi in agricultural soil with crops of different mycotrophy. *Agric. Food Sci.* **2012**, *21*, 12–27. [CrossRef]
49. R Core Team. R: A Language and Environment for Statistical Computing (Version 3.1.2). 2019. Available online: https://www.r-project.org/index.html (accessed on 21 July 2020).
50. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* **2017**, *82*. [CrossRef]
51. Bergmann, J.; Weigelt, A.; Van Der Plas, F.; Laughlin, D.C.; Kuyper, T.W.; Guerrero-Ramírez, N.R.; Valverde-Barrantes, O.J.; Bruelheide, H.; Freschet, G.T.; Iversen, C.M.; et al. The fungal collaboration gradient dominates the root economics space in plants. *Sci. Adv.* **2020**, *6*, eaba3756. [CrossRef]
52. Roumet, C.; Birouste, M.; Picon-Cochard, C.; Ghestem, M.; Osman, N.; Vrignon-Brenas, S.; Cao, K.-F.; Stokes, A. Root structure-function relationships in 74 species: Evidence of a root economics spectrum related to carbon economy. *New Phytol.* **2016**, *210*, 815–826. [CrossRef]
53. Garnier, E.; Navas, M.-L. *Diversité Fonctionnelle des Plantes: Traits des Organismes, Structure des Communautés, Propriétés des Ecosystèmes: Cours*; De Boeck: Bruxelles, Belgium, 2013.
54. Freschet, G.T.; Kichenin, E.; Wardle, D.A. Explaining within-community variation in plant biomass allocation: A balance between organ biomass and morphology above vs below ground? *J. Veg. Sci.* **2015**, *26*, 431–440. [CrossRef]
55. Comas, L.H.; Mueller, K.E.; Taylor, L.; Midford, P.E.; Callahan, H.S.; Beerling, D.J. Evolutionary Patterns and Biogeochemical Significance of Angiosperm Root Traits. *Int. J. Plant Sci.* **2012**, *173*, 584–595. [CrossRef]
56. Li, Y.; Bezemer, T.M.; Yang, J.; Lü, X.; Li, X.; Wen-Ju, L.; Han, X.; Li, Q. Changes in litter quality induced by N deposition alter soil microbial communities. *Soil Boil. Biochem.* **2019**, *130*, 33–42. [CrossRef]
57. Neely, C.L.; Beare, M.; Hargrove, W.L.; Coleman, D.C. Relationships between fungal and bacterial substrate-induced respiration, biomass and plant residue decomposition. *Soil Boil. Biochem.* **1991**, *23*, 947–954. [CrossRef]
58. Sauvadet, M.; Chauvat, M.; Brunet, N.; Bertrand, I. Can changes in litter quality drive soil fauna structure and functions? *Soil Boil. Biochem.* **2017**, *107*, 94–103. [CrossRef]
59. Grayston, S.; Griffith, G.; Mawdsley, J.; Campbell, C.D.; Bardgett, R. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Boil. Biochem.* **2001**, *33*, 533–551. [CrossRef]
60. Davison, J.; Öpik, M.; Daniell, T.J.; Moora, M.; Zobel, M. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbiol. Ecol.* **2011**, *78*, 103–115. [CrossRef]
61. Boerner, R.E.J. Plant life span and response to inoculation with vesicular-arbuscular mycorrhizal fungi. *Mycorrhiza* **1992**, *1*, 153–161. [CrossRef]
62. Liu, W. Correlation between specific fine root length and mycorrhizal colonization of maize in different soil types. *Front. Agric. China* **2009**, *3*, 13–15. [CrossRef]
63. Montgomery, D.R. Soil erosion and agricultural sustainability. *PANS* **2007**, *104*, 13268–13272. [CrossRef]
64. Gyssels, G.; Poesen, J.; Bochet, E.; Li, Y. Impact of plant roots on the resistance of soils to erosion by water: A review. *Prog. Phys. Geogr. Earth Environ.* **2005**, *29*, 189–217. [CrossRef]
65. Jastrow, J.; Miller, R. Soil Aggregate Stabilization and Carbon Sequestration: Feedbacks through Organomineral Associations. In *Soil Processes and the Carbon Cycle*; CRC Press: Boca Raton, FL, USA, 2018; pp. 207–223.

66. Sanaullah, M.; Chabbi, A.; Leifeld, J.; Bardoux, G.; Billou, D.; Rumpel, C. Decomposition and stabilization of root litter in top- and subsoil horizons: What is the difference? *Plant Soil* **2010**, *338*, 127–141. [CrossRef]
67. Wright, A.J.; Schnitzer, S.A.; A Dickie, I.; Gunderson, A.R.; Pinter, G.A.; Mangan, S.A.; Reich, P.B. Complex facilitation and competition in a temperate grassland: Loss of plant diversity and elevated CO_2 have divergent and opposite effects on oak establishment. *Oecologia* **2012**, *171*, 449–458. [CrossRef] [PubMed]
68. Noguchi, S.; Nik, A.R.; Kasran, B.; Tani, M.; Sammori, T.; Morisada, K. Soil physical properties and preferential flow pathways in tropical rain forest, Bukit Tarek, Peninsular Malaysia. *J. For. Res.* **1997**, *2*, 115–120. [CrossRef]
69. De Baets, S.; Poesen, J.; Knapen, A.; Galindo, P. Impact of root architecture on the erosion-reducing potential of roots during concentrated flow. *Earth Surf. Process. Landforms* **2007**, *32*, 1323–1345. [CrossRef]
70. Le Bissonnais, Y.; Prieto, I.; Roumet, C.; Nespoulous, J.; Metayer, J.; Huon, S.; Villatoro, M.; Stokes, A. Soil aggregate stability in Mediterranean and tropical agro-ecosystems: Effect of plant roots and soil characteristics. *Plant Soil* **2017**, *424*, 303–317. [CrossRef]
71. Fornara, D.A.; Tilman, D. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *J. Ecol.* **2008**, *96*, 314–322. [CrossRef]
72. Kätterer, T.; Bolinder, M.A.; Andrén, O.; Kirchmann, H.; Menichetti, L. Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. *Agric. Ecosyst. Environ.* **2011**, *141*, 184–192. [CrossRef]
73. Rasse, D.P.; Rumpel, C.; Dignac, M.-F. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant Soil* **2005**, *269*, 341–356. [CrossRef]
74. Freschet, G.T.; Violle, C.; Roumet, C.; Garnier, E. Interactions between Soil and Vegetation: Structure of Plant Communities and Soil Functioning. In *Soils as a Key Component of the Critical Zone 6: Ecology*; Wiley: Hoboken, NJ, USA, 2018; pp. 83–104.
75. Philippot, L.; Hallin, S.; Börjesson, G.; Baggs, E.M. Biochemical cycling in the rhizosphere having an impact on global change. *Plant Soil* **2008**, *321*, 61–81. [CrossRef]
76. Orwin, K.H.; Buckland, S.M.; Johnson, D.; Turner, B.L.; Smart, S.M.; Oakley, S.; Bardgett, R.D. Linkages of plant traits to soil properties and the functioning of temperate grassland. *J. Ecol.* **2010**, *98*, 1074–1083. [CrossRef]
77. Abalos, D.; De Deyn, G.B.; Kuyper, T.W.; Van Groenigen, J.W. Plant species identity surpasses species richness as a key driver of N2O emissions from grassland. *Glob. Chang. Boil.* **2013**, *20*, 265–275. [CrossRef] [PubMed]
78. Necpalova, M.; Anex, R.P.; Kravchenko, A.N.; Abendroth, L.J.; Del Grosso, S.J.; Dick, W.A.; Helmers, M.J.; Herzmann, D.; Lauer, J.G.; Nafziger, E.D.; et al. What does it take to detect a change in soil carbon stock? A regional comparison of minimum detectable difference and experiment duration in the north central United States. *J. Soil Water Conserv.* **2014**, *69*, 517–531. [CrossRef]
79. Jungers, J.M.; DeHaan, L.H.; Mulla, D.J.; Sheaffer, C.C.; Wyse, N.L. Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. *Agric. Ecosyst. Environ.* **2019**, *272*, 63–73. [CrossRef]
80. Sutherlin, C.E.; Brunsell, N.; De Oliveira, G.; Crews, T.E.; DeHaan, L.; Vico, G. Contrasting Physiological and Environmental Controls of Evapotranspiration over Kernza Perennial Crop, Annual Crops, and C4 and Mixed C3/C4 Grasslands. *Sustainability* **2019**, *11*, 1640. [CrossRef]
81. De Oliveira, G.; Brunsell, N.A.; Sutherlin, C.E.; Crews, T.E.; DeHaan, L.R. Energy, water and carbon exchange over a perennial Kernza wheatgrass crop. *Agric. For. Meteorol.* **2018**, *249*, 120–137. [CrossRef]
82. Poirier, V.; Angers, D.A.; Whalen, J.K. Formation of millimetric-scale aggregates and associated retention of 13C–15N-labelled residues are greater in subsoil than topsoil. *Soil Boil. Biochem.* **2014**, *75*, 45–53. [CrossRef]
83. Prieto, I.; Stokes, A.; Roumet, C. Root functional parameters predict fine root decomposability at the community level. *J. Ecol.* **2016**, *104*, 725–733. [CrossRef]
84. Freschet, G.T.; Aerts, R.; Cornelissen, J.H.C. A plant economics spectrum of litter decomposability: Afterlife effects of the plant economics spectrum. *Funct. Ecol.* **2012**, *26*, 56–65. [CrossRef]
85. Guyonnet, J.P.; Cantarel, A.; Simon, L.; Haichar, F.E.Z. Root exudation rate as functional trait involved in plant nutrient-use strategy classification. *Ecol. Evol.* **2018**, *8*, 8573–8581. [CrossRef]

86. Poorter, H.; Niklas, K.J.; Reich, P.B.; Oleksyn, J.; Poot, P.; Mommer, L. Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control: Tansley review. *New Phytol.* **2012**, *193*, 30–50. [CrossRef]
87. Huo, C.; Luo, Y.; Cheng, W. Rhizosphere priming effect: A meta-analysis. *Soil Boil. Biochem.* **2017**, *111*, 78–84. [CrossRef]
88. Barea, J.M.; Azcón, R.; Azcón-Aguilar, C. Mycorrhizosphere interactions to improve plant fitness and soil quality. *Antonie van Leeuwenhoek* **2002**, *81*, 343–351. [CrossRef] [PubMed]
89. Johansson, J.F.; Paul, L.R.; Finlay, R.D. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol. Ecol.* **2004**, *48*, 1–13. [CrossRef] [PubMed]
90. Rillig, M.C.; Wright, S.F.; Eviner, V.T. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: Comparing effects of five plant species. *Plant Soil* **2002**, *238*, 325–333. [CrossRef]
91. De Vries, F.T.; Hoffland, E.; Van Eekeren, N.; Brussaard, L.; Bloem, J. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Boil. Biochem.* **2006**, *38*, 2092–2103. [CrossRef]
92. Bardgett, R.D.; Hobbs, P.J.; Frostegård, Å. Changes in soil fungal: Bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biol. Fert. Soils* **1996**, *22*, 261–264. [CrossRef]
93. Clemmensen, K.E.; Bahr, A.; Ovaskainen, O.; Dahlberg, A.; Ekblad, A.; Wallander, H.; Stenlid, J.; Finlay, R.; Wardle, D.A.; Lindahl, B.D. Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest. *Science* **2013**, *339*, 1615–1618. [CrossRef]
94. Six, J.; Frey, S.D.; Thiet, R.K.; Batten, K.M. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. *Soil Sci. Soc. Am. J.* **2006**, *70*, 555–569. [CrossRef]
95. Cotrufo, M.F.; Soong, J.L.; Horton, A.J.; Campbell, E.E.; Haddix, M.L.; Wall, D.H.; Parton, W.J. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat. Geosci.* **2015**, *8*, 776–779. [CrossRef]
96. Haddix, M.L.; Paul, E.A.; Cotrufo, M.F. Dual, differential isotope labeling shows the preferential movement of labile plant constituents into mineral-bonded soil organic matter. *Glob. Chang. Boil.* **2016**, *22*, 2301–2312. [CrossRef] [PubMed]
97. Lemaire, G.; Gastal, F.; Franzluebbers, A.J.; Chabbi, A. Grassland–Cropping Rotations: An Avenue for Agricultural Diversification to Reconcile High Production with Environmental Quality. *Environ. Manag.* **2015**, *56*, 1065–1077. [CrossRef] [PubMed]
98. Grover, K.; Karsten, H.D.; Roth, G.W. Corn Grain Yields and Yield Stability in Four Long-Term Cropping Systems. *Agron. J.* **2009**, *101*, 940–946. [CrossRef]
99. Franzluebbers, A.J.; Sawchik, J.; Taboada, M.A. Agronomic and environmental impacts of pasture–crop rotations in temperate North and South America. *Agric. Ecosyst. Environ.* **2014**, *190*, 18–26. [CrossRef]
100. King, A.E.; Blesh, J. Crop rotations for increased soil carbon: Perenniality as a guiding principle. *Ecol. Appl.* **2017**, *28*, 249–261. [CrossRef] [PubMed]
101. Soussana, J.-F.; Lemaire, G. Coupling carbon and nitrogen cycles for environmentally sustainable intensification of grasslands and crop-livestock systems. *Agric. Ecosyst. Environ.* **2014**, *190*, 9–17. [CrossRef]
102. Crews, T.E.; Peoples, M.B. Can the Synchrony of Nitrogen Supply and Crop Demand be Improved in Legume and Fertilizer-based Agroecosystems? A Review. *Nutr. Cycl. Agroecosystems* **2005**, *72*, 101–120. [CrossRef]