agronomy

MDPI

Communication

Crop Conversion from Annual to Perennials: An Effective Strategy to Affect Soil Multifunctionality

Panpan Liu [1,2], Dong Wang [1,*], Yue Li [3], Ji Liu [2], Yongxing Cui [4], Guopeng Liang [5], Chaoqun Wang [6,7], Chao Wang [8], Daryl L. Moorhead [9] and Ji Chen [2,10,*]

1 International Joint Research Laboratory of Global Change Ecology, School of Life Sciences, Henan University, Kaifeng 475004, China; liupanpan199404@163.com
2 State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an 710061, China
3 College of Ecology, Lanzhou University, Lanzhou 730000, China
4 Institute of Biology, Freie Universität Berlin, 14195 Berlin, Germany
5 Department of Forest Resources, University of Minnesota, Saint Paul, MN 55108, USA
6 Biogeochemistry of Agroecosystems, University of Göttingen, 37077 Göttingen, Germany
7 Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC V6S0K4, Canada
8 School of Ecology and Environment, Northwestern Polytechnical University, Xi'an 710072, China
9 Department of Environmental Sciences, University of Toledo, Toledo, OH 43606, USA
10 Department of Agroecology, Aarhus University, 8830 Tjele, Denmark
* Correspondence: wangdong19882005@163.com (D.W.); ji.chen@agro.au.dk (J.C.)

Citation: Liu, P.; Wang, D.; Li, Y.; Liu, J.; Cui, Y.; Liang, G.; Wang, C.; Wang, C.; Moorhead, D.L.; Chen, J. Crop Conversion from Annual to Perennials: An Effective Strategy to Affect Soil Multifunctionality. *Agronomy* **2024**, *14*, 594. https://doi.org/10.3390/agronomy14030594

Academic Editors: Nicolai David Jablonowski, Elżbieta Harasim and Cezary A. Kwiatkowski

Received: 18 January 2024
Revised: 29 February 2024
Accepted: 13 March 2024
Published: 15 March 2024

Correction Statement: This article has been republished with a minor change. The change does not affect the scientific content of the article and further details are available within the backmatter of the website version of this article.

Abstract: Although crop conversion from annual to perennial crops has been considered as one path towards climate-smart and resource-efficient agriculture, the effects of this conversion on soil multifunctionality and biomass yields remain unclear. The objective of the study is to enhance soil multifunctionality while exerting a marginal influence on farmer income. Here, we investigated the effects of annual winter wheat (*Triticum aestivum* L.) and two perennial crops (a grass (*Lolium perenne* L.), a legume (*Medicago sativa* L.), and their mixture) on soil multifunctionality and biomass yield on the Yellow River floodplain. Soil multifunctionality was assessed by the capacity of water regulation and the multifunctionality of carbon (C), nitrogen (N), and phosphorus (P) cycles. C cycle multifunctionality index is the average of β-xylosidase, β-cellobiosidase, and β-1, 4-glucosidase. N cycle multifunctionality index is the average of L-leucine aminopeptidase and β-1, 4-N-acetyl-glucosaminidase, and acid phosphatase represented (and dominated) P cycle functions. The results showed that perennial crops enhanced soil multifunctionality by 207% for *L. perenne*, 311% for *M. sativa*, and 438% for *L. perenne* + *M. sativa*, compared with annual winter wheat (*T. aestivum*). The effect of perennial crops on soil multifunctionality increased with infiltration rate, dissolved organic C, microbial biomass C, and extracellular enzymatic activities for both C and N acquisition. However, we observed that perennial crops had a lower biomass yield than annual crop. Therefore, the transition of agricultural landscapes to perennials needs to take into account the balance between environmental protection and food security, as well as environmental heterogeneity, to promote sustainable agricultural development.

Keywords: land use change; Yellow River floodplain; crop type; soil extracellular enzymes; soil functions; annual and perennial crops

1. Introduction

Annual and perennial crops supply a multitude of functions and services in agroecosystems. Annual crops provide around 80% of the global food for humans [1], which is achieved through intensive management such as mechanized tillage, fertilization, and use of pesticides, in addition to the expansion of cropping areas [2]. However, these intensive agronomic practices are now recognized as having negative impacts on soil health such as aggravating soil erosion [3], reducing soil fertility [4,5], and impairing multiple ecosystem functions and services, that is, soil multifunctionality [6,7]. The purpose of quantifying soil multifunctionality is to assess the capacity of soil to concurrently fulfill multiple functions [8], such as water regulation and soil carbon (C), nitrogen (N), and phosphorus (P) cycle multifunctionality. The initial step of the methodology involves delineating the soil function and selecting the pertinent soil parameters. Subsequently, the soil multifunctionality index is always computed [8] by the averaging approach. The soil multifunctionality is fundamental to soil health and represents a comprehensive manifestation of soil well-being. In agroecosystems, the understanding of soil function has evolved beyond mere maximization of biomass yield, encompassing a comprehensive pursuit of coordinated objectives such as optimizing yield, enhancing soil quality, promoting environmental sustainability, and improving resource efficiency. Furthermore, the adjustment of Chinese dietary habits has emphasized the growing importance of animal husbandry, thus elevating the prominence of perennial herbs [9]. Simultaneously, the suboptimal soil quality in the Yellow River floodplain impedes the productivity of conventional agriculture.

Given these negative impacts of annual crops on the environment, such as aggravating soil erosion, reducing soil fertility, and impairing multiple ecosystem functions and services, perennial crops are increasingly suggested as an alternative option to maintain soil multifunctionality and biomass yield [10]. Perennial crops can play an integral role in increasing soil multifunctionality and biomass yields [11,12]. For example, decreasing tillage frequency [13] can retain biomass yield [14] and increase soil multifunctionality [11]. Thus, perennial crops can provide an alternative management strategy to intensive annual crops. However, the impacts of crop conversion from annual to perennial crops on soil multifunctionality and biomass yield are unclear. Furthermore, the extent to which these impacts depend on the specific perennial crops remains unknown.

Numerous studies have suggested that the conversion of annual crops to perennial crops had significant impacts on soil multifunctionality [15]. Generally, grasses with fibrous and rhizomatous roots densely grow near the soil surface, forming a network that reduces soil pore space, restricts water movement, and reduces soil infiltration rate [16,17]. This phenomenon contributes to improved physical properties of the soil such as total porosity and water holding capacity [18]. Moreover, leguminous plants with robust taproots and N-fixing capabilities can enhance the physicochemical properties of the soil by increasing extracellular enzymatic activities (EEAs), thereby influencing C, N, and P cycles [19,20]. The mixed sowing of legume–grass combinations exhibits complementary rather than competitive potential in terms of growth morphology and development rhythm, utilization of soil nutrients, as well as root distributions [21,22]. However, there are few reports on the effect of a single sowing of grass, legume, or mixed legume–grass combinations on soil multifunctionality.

Here, we conducted a one-year field experiment involving crop type conversion in the Yellow River floodplain, China, to explore the influences of implementing two different perennial crops on soil multifunctionality and biomass yield. The Yellow River floodplain is an important agroecosystem of North China, and serves as the main region of grain production in China. The soil multifunctionality was assessed by measuring soil physical, chemical, and microbial properties and soil EEAs. The Z-score average of eight soil variables related to water regulation and C, N, and P cycling was calculated (details in the Materials and Methods). Our research questions were the following: (1) Does the soil multifunctionality respond to annual and perennial (a grass, a legume, and a mix of these both) crops differently? (2) What are the relationships between soil multifunctionality and biomass yield under crop conversion from annual to perennial crops?

2. Materials and Methods

2.1. Study Site and Experimental Design

The field experiment was located in the Yellow River floodplain in Henan Province, China (34°66′ N, 114°23′ E; 76 m above sea level) (Figure 1). Based on the nearby meteorological data records at the site, the average annual temperature is 14.5 °C, and the mean annual precipitation is 627 mm. The soil is classified as Aquic Inceptisol (WRB classification), a typical alkaline soil in the North China Plain derived from alluvial sediments of the Yellow River [23]. At the initial stage of the study, the surface soil at 0–20 cm depth exhibited a soil bulk density of 1.59 g cm^{-3}, a total C content of 13.20 g kg^{-1}, a total N content of 0.82 g kg^{-1}, a total P content of 0.88 g kg^{-1}, an available P content of 37.5 mg kg^{-1}, and an available N content of 11.2 mg kg^{-1}. The pH of the soil was 8.23. The soil within the study area exhibited a composition of 0.70% clay, 39.09% silt, and 60.22% sand [24]. Before establishing the field experiment, there was at least a 20-year history of rotation to produce winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* ssp. *mays*), which were common crops for the Yellow River floodplain.

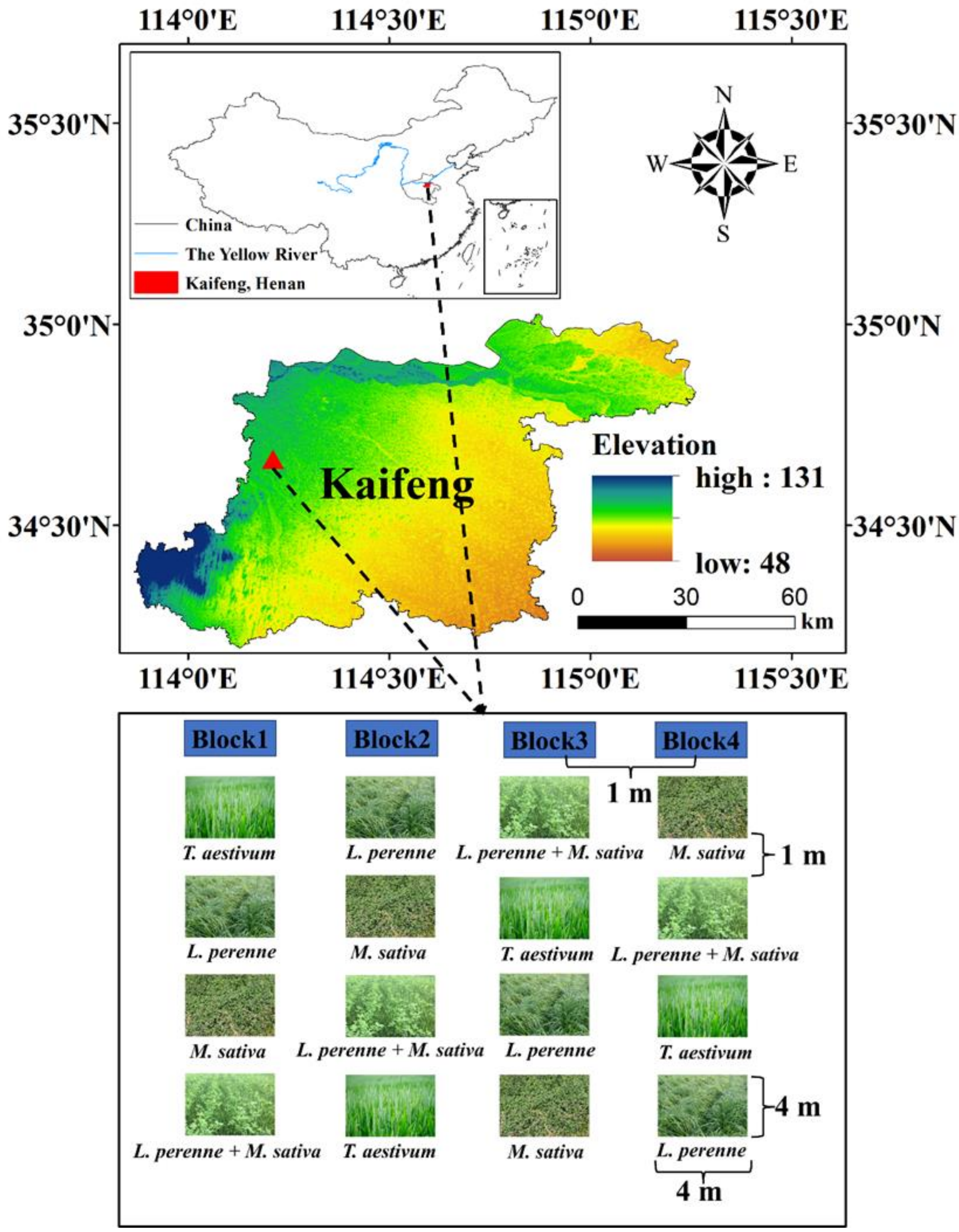

Figure 1. Location of the study area in Kaifeng, Henan Province, China. The distribution of all experiment plots. *Triticum aestivum* L., *Lolium perenne* L., *Medicago sativa* L., and *L. perenne* + *M. sativa*.

The experimental was established in 2020, and designed to compare biomass yield of various crops and soil multifunctionality (SMF). The investigated crop types were annual winter wheat, and the perennials, *Lolium perenne* L., *Medicago sativa* L., and *L. perenne* + *M. sativa*. The crops of annual winter wheat, *L. perenne*, and *M. sativa* were manually planted using a drill with a sowing capacity of 30.0, 22.5, and 30.0 kg per ha and a row spacing of 15 cm, relatively (Changjing Garden Company, Chian). The planting ratio of *L. perenne* + *M. sativa* is 1:1. The total experimental area was about 400 m^2 and arranged in a split-plot design with four blocks per crop. The crop type split-plots consisted of *L. perenne*, *M. sativa*, a mixture of *L. perenne* + *M. sativa*, and a control treatment consisting of the annual winter wheat, and there was at least one meter buffer zones between adjacent split-plots. The size of the blocks was 4 m by 20 m. The split-plot size was 4 m by 4 m (Figure 1).

2.2. Soil Sample Collection

In July 2021, the samples in each plot were collected with five soil cores at 0–10 cm and 10–20 cm depths and mixed to produce a composite soil sample at each depth. Samples were placed on ice in a cooler and transported back to the laboratory immediately after the field sampling. All soil samples were sieved using a 2 mm mesh in the lab [25]. Each sample was separated into three portions. The first portion was air-dried to measure physical properties. The second portion was for chemical properties. The third portion was stored at 4 °C for the soil EEAs and microbial biomass analysis.

2.3. Measurements

2.3.1. Biomass Yield

The annual biomass yield of winter wheat was measured by harvesting an area of 1 m × 1 m from the middle of each plot when the crop reached physiological maturity. All perennial crops were collected in July 2021, when the plant community biomass peaked. One 1 m × 1 m quadrat was selected randomly in each plot at harvest to determine perennial biomass yields. The dry weight of the plant material was measured after oven-drying at 65 °C for 48 h [26].

2.3.2. Soil Physical, Chemical, and Microbial Properties

Soil steady infiltration rate was measured by the point source method [27]. Soil water content was determined by the drying method. Soil bulk density was measured using the metal ring (100 cm^3) method. Soil total porosity and water-holding capacity were measured and calculated by the method and equations proposed by Huang et al. (2019) [28]. Soil pH was measured in all of the soil samples by a pH electrode in a mixture of soil and water, with a soil/water ratio of 1:2.5 (Sartorius Basic PH Meter PB-10, Göttingen, Germany). Dissolved organic C in the extracts was detected using a multi N/C 2100S TOC-TN analyzer (Analytik Jena AG, Jena, Germany). Soil microbial biomass C and N were measured by the chloroform fumigation extraction method with conversion factors of 0.45 and 0.54 used to calculate microbial biomass C and N [29].

2.3.3. Soil Extracellular Enzymatic Activities

Soil EEAs including β-xylosidase (BX), β-cellobiosidase (CBH), and β-1, 4-glucosidase (BG), β-1, 4-N-acetyl-glucosaminidase (NAG), L-leucine aminopeptidase (LAP), and acid phosphatase (AP) were measured from 1 g of soil using fluorometry microplates [30]. These soil EEAs were identified using 4-methyl-umbelliferone and 7-amino-4-methylcoumarin [31]. Soil EEAs were expressed in units of nmol activity g^{-1} dry soil h^{-1} (nmol g^{-1} h^{-1}) Enzyme active substrate (Thermo Fisher SCIENTIFIC, Shanghai, China).

To estimate the C (BG, CBH, and BX)-, N (NAG and LAP)-, and P-related enzymes (AP), we used an equation to normalize the soil EEAs performing similar functions [32,33]. Let us take the calculation of C acquisition (C-acq) EEAs as an example:

$$\text{C-acq} = \sqrt[3]{(\text{BG}\cdot\text{BX}\cdot\text{CBH})}, \tag{1}$$

2.3.4. Assessing Soil Multifunctionality

The SMF is not a one-dimensional measurable process, and includes quantifying the provision of multiple ecosystem processes and services simultaneously [34]. We evaluated multifunctionality using the SMF index, obtained as the average of several functions. The index is an averaging approach and attempts to combine a collection of soil functions into a single index, so that high values of SMF mean high values of many, but not necessarily all, of the functions included.

To obtain the SMF index for each plot, we first normalized each of the two soil function categories measured (water regulation and nutrient cycle functions) using the Z-score transformation. The process of nutrient cycling encompasses the processes of carbon, nitrogen, and phosphorus cycling [35]. These standardized soil functions were then averaged to obtain an SMF index [11,36]. Water regulation was composed of total porosity and water-holding capacity [18]. Carbon (C) cycle multifunctionality was composed of BX, BG, and CBH activities. N cycle multifunctionality was composed of NAG and LAP activities. P cycle multifunctionality was composed of AP activity. The Z-score transformation was used to standardize soil EEAs to acquire a multifunctionality index based on the methods described by Delgado-Baquerizo et al. (2016) [34].

$$\text{Z-score} = (\mathrm{x} - \mathrm{mean_i})/\mathrm{SD_i} \quad (2)$$

where x is the measured soil EEAs, mean is the average of enzyme i, and SD is the standard deviation of enzyme i.

2.4. Statistical Analysis

Statistical analyses were performed in R (version 4.1.3) by using packages nlme [37] and ggiraphExtra [38]. All observational data were first tested for normality using the Kolmogorov–Smirnov method, and the non-normally distributed data were logarithm- or square root-transformed. Linear mixed-effects models were performed to tested the effects of crop types and soil depth on soil properties including soil water content, bulk density, steady infiltration rate, soil pH, dissolved organic C, microbial biomass C, and microbial biomass N. The block in the experiment was considered as a random effect and crop types and soil depths as fixed effect. The effects of crop types on the eight measurable proxies, the three calculated proxies, and the five multifunctionality indexes were analyzed in linear mixed models, with crop types as a fixed factor. Between-group comparisons were performed using Tukey's post hoc tests. Pearson correlations were used to assess the relationships between SMF, biomass, and soil properties. The comprehensive analysis between SMF and biomass yield was realized with the "ggiraphExtra" package.

3. Results

3.1. Soil Physical, Chemical, and Microbial Properties

Compared with an annual crop of winter wheat, perennial crops significantly affected soil physical, chemical, and microbial properties (Table S1; Figures 2 and 3). The main effects of perennial crops on soil properties were limited to the topsoil with the single exception of bulk density in both topsoil and subsoil (Table S1). Compared with an annual crop of winter wheat, perennial crops (*L. perenne*, *M. sativa* and *L. perenne* + *M. sativa*) significantly increased the soil water content by 47%, 40%, and 38% (Table S1; Figure 2), steady infiltration rate by 18.4 mm h^{-1}, 26.7 mm h^{-1}, and 22.8 mm h^{-1} (Figure 3), dissolved organic C by 40.1 mg kg^{-1}, 28.4 mg kg^{-1}, and 52.0 mg kg^{-1} (Table S1; Figure 2), and microbial biomass C by 89.0 mg kg^{-1}, 63.1 mg kg^{-1}, and 116 mg kg^{-1}, respectively (Table S1; Figure 2).

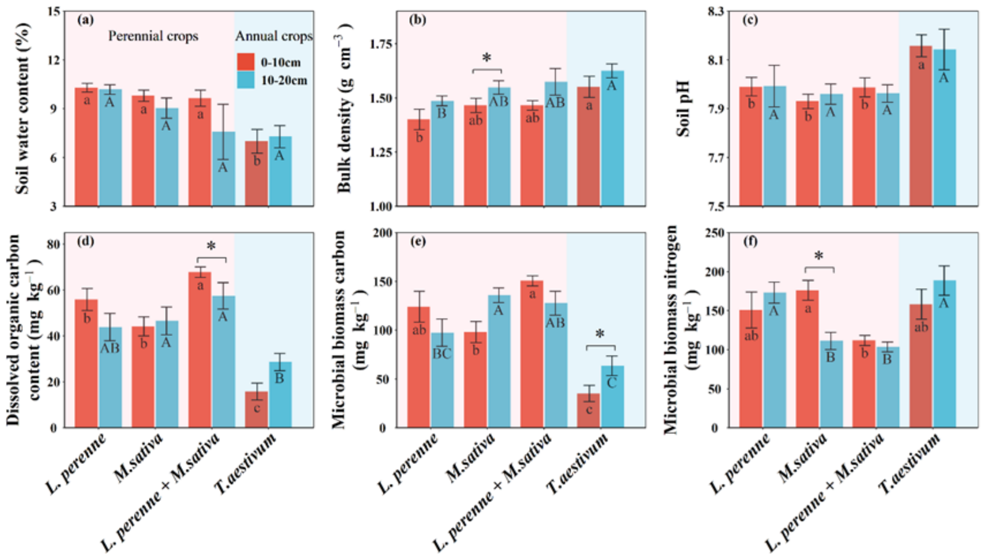

Figure 2. Mean values are shown with standard errors for (**a**) soil water content, (**b**) bulk density, (**c**) soil pH, (**d**) dissolved organic carbon content, (**e**) microbial biomass carbon, and (**f**) microbial biomass nitrogen in each crop type and soil depth. Lowercase and uppercase letters denote significant differences at $p < 0.05$ between crop types within 0–10 cm and 10–20 cm, respectively. Asterisks denote significant differences between 0 and 10 cm and between 10 and 20 cm within each crop type at $p < 0.05$.

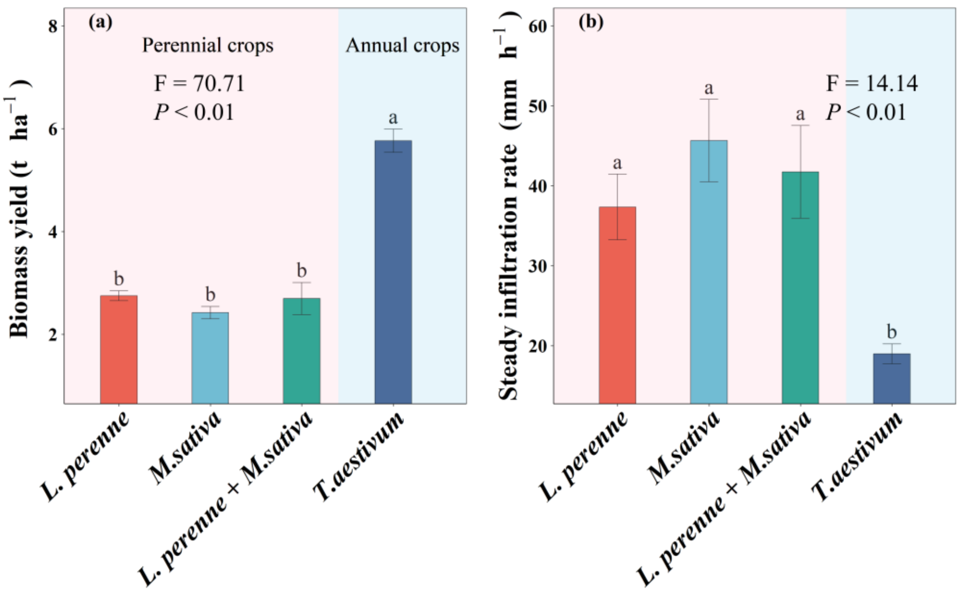

Figure 3. Mean values are shown with standard errors for (**a**) biomass yield and (**b**) steady infiltration rate in each crop type. Note: different letters in the bars indicate significant differences among four crop types according to Tukey's HSD tests ($p < 0.05$).

3.2. Soil Extracellular Enzymatic Activities

Compared with an annual crop of winter wheat, perennial crops (*L. perenne*, *M. sativa* and *L. perenne* + *M. sativa*) significantly increased C-acq EEAs by 6.09 nmol g^{-1} h^{-1}, 5.19 nmol g^{-1} h^{-1}, and 10.6 nmol g^{-1} h^{-1} (Figure 4), and N-acq EEAs by 2.57 nmol g^{-1} h^{-1}, 1.40 nmol g^{-1} h^{-1}, and 2.72 nmol g^{-1} h^{-1}, respectively (Figure 4). Perennial crops (*M. sativa* and *L. perenne* + *M. sativa*) also significantly increased P-acq EEAs by 61.5 nmol g^{-1} h^{-1} and 51.6 nmol g^{-1} h^{-1}, respectively (Figure 4). The soil depth only significantly affected C-acq and P-acq EEAs (Table S2).

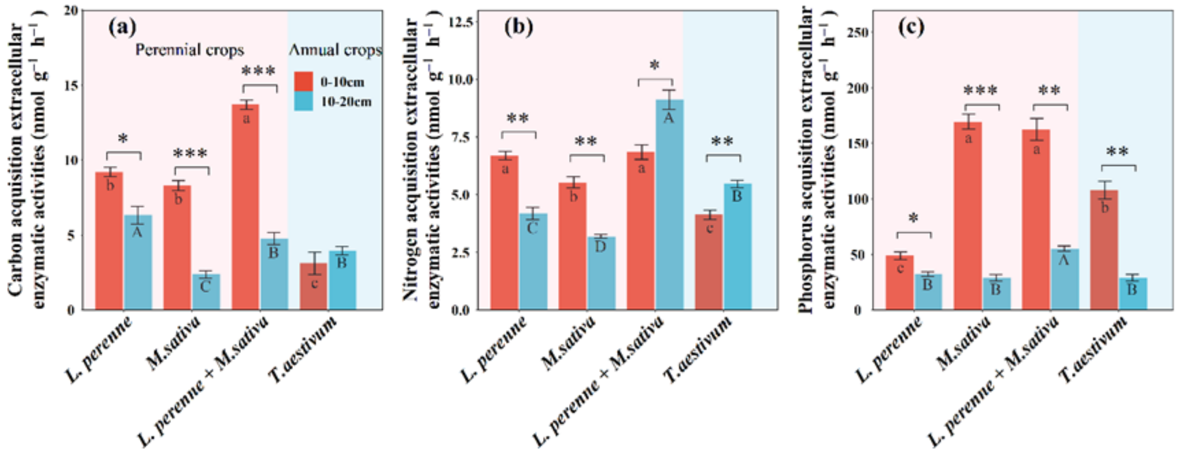

Figure 4. Mean values are shown with standard errors for (**a**) carbon acquisition extracellular enzymatic activities, (**b**) nitrogen acquisition extracellular enzymatic activities, and (**c**) phosphorus acquisition extracellular enzymatic activities in each crop type and soil depth. Lowercase and uppercase letters denote significant differences at $p < 0.05$ between crop types within 0–10 cm and 10–20 cm, respectively. One, two, and three asterisks denote significant differences between 0 and 10 cm and between 10 and 20 cm within each crop type at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

3.3. Soil Multifunctionality and Biomass Yield

Compared with an annual crop of winter wheat, perennial crops (*L. perenne*, *M. sativa* and *L. perenne* + *M. sativa*) significantly enhanced the SMF by 207%, 311%, and 438% (Figure 5), C cycle multifunctionality by 188%, 151%, and 318% (Figure 6), and N cycle multifunctionality by 170%, 100%, and 150% (Figure 6). The two combined perennial crops (*M. sativa* and *L. perenne* + *M. sativa*) significantly increased the P cycle multifunctionality by 214% and 190% (Figure 6). In addition, the soil depth significantly affected the water regulation, C cycle multifunctionality, and P cycle multifunctionality (Table S3).

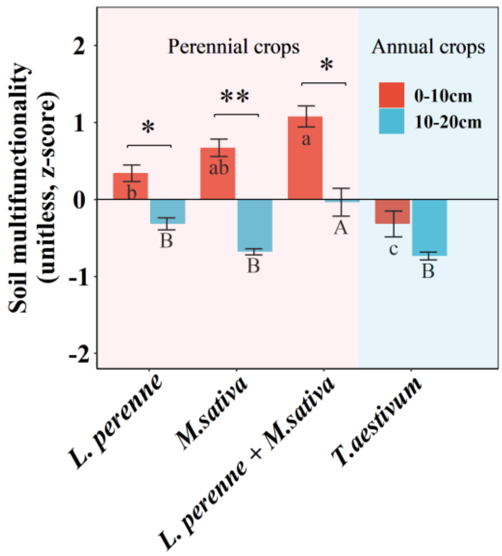

Figure 5. Mean values are shown with standard errors for soil multifunctionality index in each crop type and soil depth. Lowercase and uppercase letters denote significant differences at $p < 0.05$ between crop types within 0–10 cm and 10–20 cm, respectively. One and two asterisks denote significant differences between 0 and 10 cm and between 10 and 20 cm within each crop type at $p < 0.05$ and $p < 0.01$, respectively.

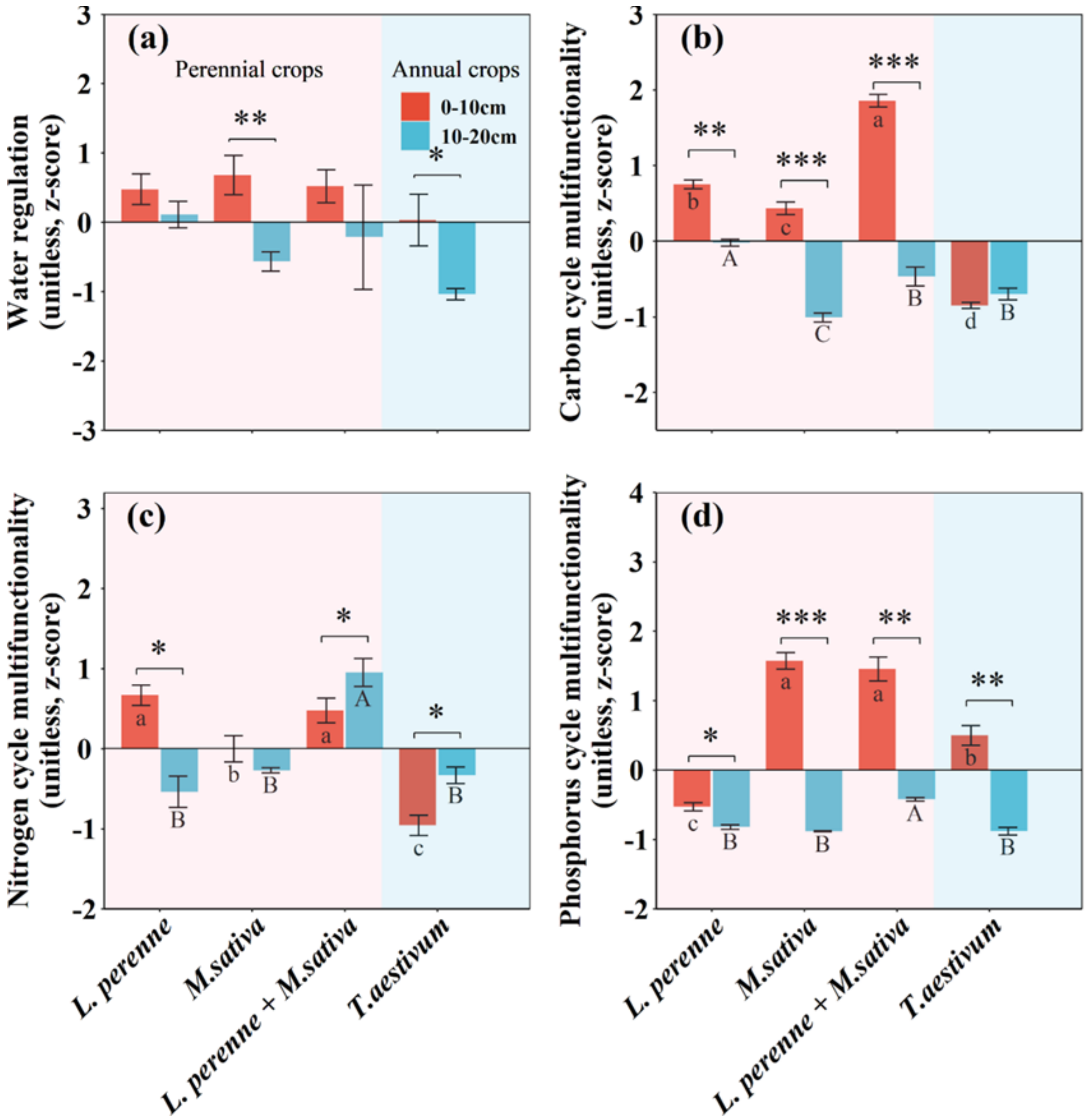

Figure 6. Mean values are shown with standard errors for (**a**) soil water regulation, (**b**) carbon cycle multifunctionality index, (**c**) nitrogen cycle multifunctionality index, and (**d**) phosphorus cycle multifunctionality index in each crop type and soil depth. Lowercase and uppercase letters denote significant differences at $p < 0.05$ between crop types within 0–10 cm and 10–20 cm, respectively. One, two, and three asterisks denote significant differences between 0–10 cm and 10–20 cm within each crop type at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

In contrast, the biomass yield was significantly lower in perennial crops (*L. perenne*, *M. sativa* and *L. perenne* + *M. sativa*) than in annual crops of winter wheat, accounting for 3.02 t ha^{-1}, 3.35 t ha^{-1}, and 2.81 t ha^{-1}, respectively (Figure 3). The perennial crop combination of *L. perenne* + *M. sativa* had the highest SMF and C cycle multifunctionality, whereas the perennial crops of *L. perenne* had the highest N cycle multifunctionality, and *M. sativa* had the highest water regulation and P cycle multifunctionality (Figure 7).

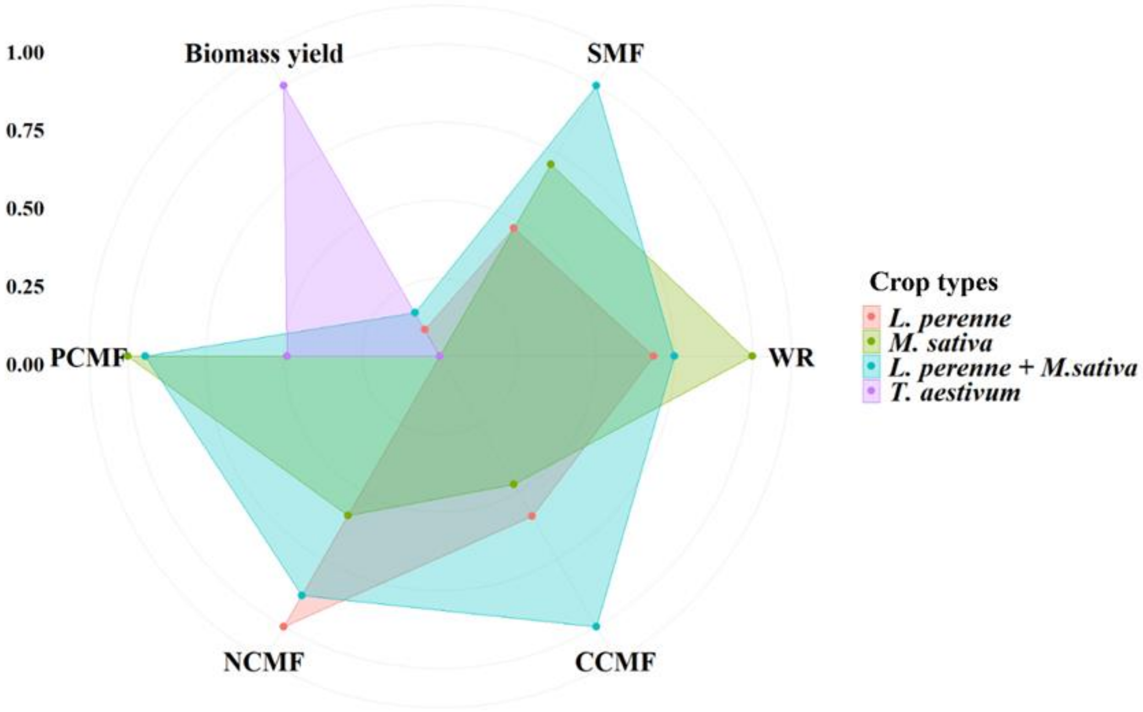

Figure 7. Soil multifunctionality (SMF), water regulation (WR), carbon cycle multifunctionality (CCMF), nitrogen cycle multifunctionality (NCMF), phosphorus cycle multifunctionality (PCMF), and biomass yield for the *T. aestivum*, *L. perenne*, *M. sativa*, and *L. perenne* + *M. sativa*. To facilitate comparison among the four crop types, the data were subjected to a standardization process to allow for direct comparison.

3.4. Correlations of Soil Multifunctionality and Biomass with Soil Properties

In general, the SMF was positively correlated with multiple indexes related to steady infiltration rate (r = 0.71, $p < 0.01$), dissolved organic C (r = 0.71, $p < 0.01$), microbial biomass C (r = 0.71, $p < 0.01$), C-acq EEAs (r = 0.804, $p < 0.001$), and N-acq EEAs (r = 0.65, $p < 0.01$) in topsoil (Figure 8a). The carbon (C) cycle multifunctionality was positively correlated with soil water content (r = 0.63, $p < 0.01$), steady infiltration rate (r = 0.62, $p < 0.05$), dissolved organic C (r = 0.895, $p < 0.001$), microbial biomass C (r = 0.882, $p < 0.001$), and N-acq EEAs (r = 0.853, $p < 0.001$). Meanwhile, the N cycle multifunctionality was positively correlated with the soil water content (r = 0.68, $p < 0.01$), steady infiltration rate (r = 0.62, $p < 0.05$), dissolved organic C (r = 0.768, $p < 0.001$), microbial biomass C (r = 0.771, $p < 0.001$), C-acq EEAs (r = 0.747, $p < 0.001$). The SMF was negatively correlated with soil pH (r = −0.66, $p < 0.01$), which was attributed to the negative correlation of the C cycle multifunctionality and N cycle multifunctionality with the soil pH (r = −0.56, $p < 0.05$, r = −0.63, $p < 0.01$). Water regulation was negatively correlated with the bulk density (r = −0.71, $p < 0.01$). There was no clear relationship between the SMF and soil water content, bulk density, microbial biomass N, and P-acq EEAs. The biomass yield showed significant positive correlations with the soil pH (r = 0.74, $p < 0.01$), and negative correlations with the soil water content (r = −0.804, $p < 0.001$), steady infiltration rate (r = −0.73, $p < 0.01$), dissolved organic C (r = −0.752, $p < 0.001$), microbial biomass C (r = −0.73, $p < 0.01$), C-acq EEAs (r = −0.742, $p < 0.001$), and N-acq EEAs (r = −0.747, $p < 0.001$) (Figure 8a).

In the subsoil, the SMF was positively correlated with the N-acq EEAs (r = 0.68, $p < 0.01$) and P-acq EEAs (r = 0.68, $p < 0.01$), which likely accounts for the observed positive correlations between the N cycle multifunctionality and P-acq EEAs (r = 0.882, $p < 0.001$), as well as the P cycle multifunctionality and N-acq EEAs (r = 0.880, $p < 0.001$) (Figure 8b).

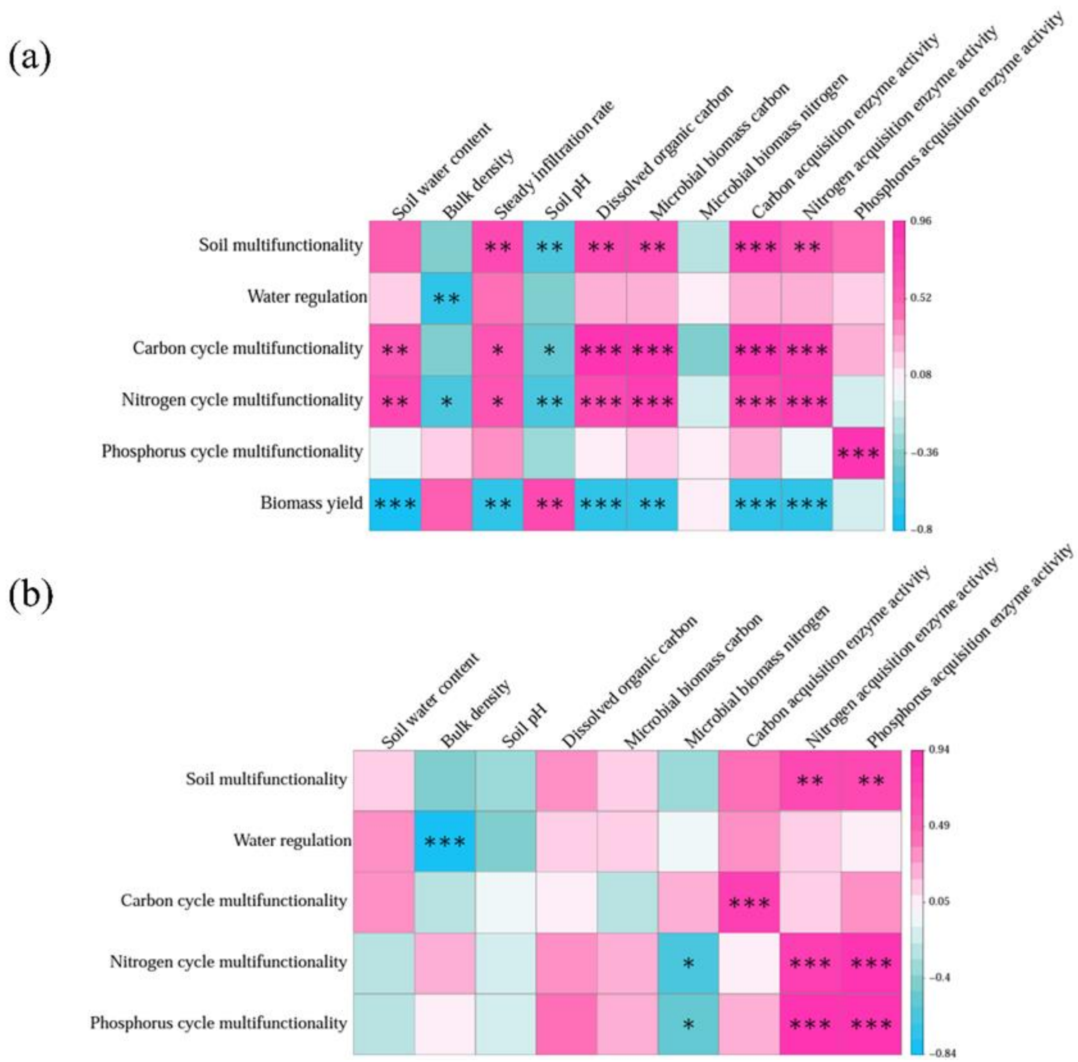

Figure 8. Pearson's correlation matrix among soil multifunctionality, biomass, soil properties, and soil enzyme activities at (**a**) 0–10 cm and (**b**) 10–20 cm soil depth. Blue color represents negative correlation; pink color represents positive correlation, significance is indicated by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

4. Discussion

We found that perennial crops significantly increased soil multifunctionality, but had varied effects on single soil functions (Figures 5 and 6). Soil multifunctionality reflected a comprehensive response of all single functions, which might show positive, negative, or non-significant responses [39]. This report suggests that the effects of perennial crops on single soil functions were greater in topsoil than in subsoil (Figure 6). Moreover, the soil steady infiltration rate, pH, dissolved organic C, microbial biomass C, and both C-acq and N-acq EEAs were positively correlated with soil multifunctionality [40], but negatively correlated with biomass yield (Figure 8a). Therefore, perennial crops, particularly mixed perennial crops, could be a promising strategy for increasing soil multifunctionality in the Yellow River floodplain but at a cost to crop yield.

4.1. Perennial Crops Increased Single Functions and Soil Multifunctionality

Soil properties and EEAs measured in this study contributed to the effects of perennial crops on C, N, P cycling multifunctionality and soil multifunctionality, but they depended strongly on the different crops. We found that the introduction of perennial crops resulted in an increase in soil multifunctionality as well as C and N cycling multifunctionality

(Figures 5 and 6). Our results were consistent with earlier findings that complementary effects of functionally for perennial crops (a grass and a legume) promoted soil multifunctionality [41]. Therefore, the incorporation of a combination of grass and legume species might represent an optimal choice for fostering sustainable development in this agroecosystem.

Compared with an annual crop of winter wheat, the perennial grass, the legume, and a mixture thereof increased overall increased overall soil multifunctionality by enhancing C and N cycle multifunctionality. For instance, the synergistic effects of distinct single functions (such as C and N cycle multifunctionality) could mutually reinforce each other, resulting in higher soil multifunctionality [42]. Indeed, perennial grasses, legumes, and mixed crops of grasses–legumes may facilitate C cycling by increasing dissolved organic C content, microbial biomass C, and C-acq EEAs, thereby increasing C cycle multifunctionality [11,32]. Moreover, perennial grasses, legumes, and mixed crops of grasses–legumes might increase microbial activity and N-acq EEAs in response to increasing dissolved organic C content, thereby increasing N cycle multifunctionality [43]. The negative correlation between water regulation and soil bulk density could counterbalance the positive effects of increased soil infiltration on water regulation (Figure 8a). These results highlight the crucial role of soil water holding capacity in water regulation, suggesting its importance over other factors [18]. In other words, regardless of whether it pertains to annual or perennial crops, the immediate impact on water regulation remains conspicuous. Our findings also suggested that P-acq EEAs play a pivotal role in distinguishing P cycle multifunctionality across different crop types [44]. The limited P-acq EEAs of perennial grasses resulted in reduced P cycle multifunctionality. Furthermore, our study underscored the importance of a neutral soil pH because it enhances soil multifunctionality [45]. Compared with annual crops, perennial (grasses, legumes, and mixed crops of grasses–legumes) crops exhibited the potential to maintain a neutral soil pH. Therefore, perennial mixed crops of grasses–legumes exhibit high potential in sustaining soil multifunctionality.

4.2. Relationship between Soil Multifunctionality and Biomass

Crop types underlay the observed relationship between soil multifunctionality and biomass yield. As shown in Figure 7, biomass yield was significantly higher in annual crops of winter wheat compared with the perennial grass, legume, and their mixture, although soil multifunctionality was higher in perennial crops. We hypothesize that there are three potential factors contributing to this result. Firstly, the growth duration of winter wheat in the Yellow River floodplain ranges from 220 to 270 days; one possible explanation is the shorter life cycle and higher rates of photosynthesis and water use efficiency observed in winter wheat compared with perennial crops [46]. Furthermore, aboveground perennial mixed crops of grasses–legumes might intensify competition for light to support photosynthesis and water use efficiency [47]. Secondly, the biomass yields of perennial crops is merely 2–4 t ha^{-1}, due to the limited duration of planting in this study, perennial crops might exhibit underdeveloped above-ground structures; it was noteworthy that the C cycle multifunctionality was significantly enhanced by 151–318% and the N cycle multifunctionality was improved by 100–170% when comparing perennial crops to annual winter wheat, and that root growth exerts a substantial influence on soil dynamics, particularly in relation to C and N cycling [48]. The roots of mixed crops of grasses–legumes enhanced soil porosity by 8% through increased growth [49] and contributed to soil organic C content through growth and turnover [45]. Thirdly, perennial crops had been found to enhance the diversity of soil microbial communities, which had demonstrated a significant positive correlation with soil multifunctionality in previous research studies [10]. Perennial crops also enhanced soil multifunctionality by fostering bacterial and fungal diversity through root development [50]. Consequently, optimizing soil multifunctionality and biomass yield should be further prioritized for sustainable agriculture in the future.

4.3. Implications and Uncertainties

Our findings have important implications for understanding the impacts of perennial crops on soil multifunctionality in the short term. For instance, perennial grasses, legumes, and mixed crops of grasses–legumes led to an increase in soil multifunctionality at various levels even after one growing cycle (Figure 7). Our results are consistent with earlier findings showing that the maintenance of soil multifunctionality is contingent upon species diversity [41]. Importantly, mixed perennial crops had the greatest effects in enhancing the soil multifunctionality and C cycle multifunctionality by significantly augmenting soil C input and EEAs. These findings were also consistent with previous research, indicating that it should be "compared with the perennial grass, legume, and their mixture not only yields a greater quantity of matter (not significant) but also provide higher energy output [51]. However, the selection of mixed perennial crops should consider not only the complementary effects but also the potential for interspecific competition.

Here, we used data from a one-year experiment as a case study to assess soil multifunctionality during initial crop conversion from annual to perennial crops. The significance of our analysis lies in its demonstration of quantifiable short-term effects on soil multifunctionality. It is crucial to acknowledge that we did not assign weights to individual functions, assuming equivalent importance for all functions [52]. Also, the perennial crops investigated in this study are not representative of all perennial crop types. In further research, we propose integrating additional functions, comparing a wider range of crop types, and seeking crops that could simultaneously enhance both soil multifunctionality and biomass yield in the long term. Unfortunately, the data in this study are limited to a one-year experiment, and it is imperative that our findings undergo further validation over an extended duration of time. The intriguing nature of our short-term findings may potentially be attributed to prompt reactions to regional climate conditions or analogous factors.

5. Conclusions

Our findings provide experimental evidence that the selected perennial crops could increase soil multifunctionality compared to an annual crop in the short-term. Perennial crops were effective for increasing soil multifunctionality in an agroecological system but led to significantly lower yields than the annual crop. Mixed perennial crops have higher potential to increase soil multifunctionality than single perennial crops. Finally, our results suggested that perennial crops have important implications for promoting soil functions of the agroecological system in the Yellow River floodplain. In addition, more research on potential environmental impacts is required before applying this knowledge across broad areas. Although long-term and extensive research is still required to approve the presented results obtained after one year only on the long-term, and to optimize the selection of ideal annual and perennial crops, our results provide early insight by underscoring the need and the benefits of systematic research on crop-specific biomass yield and soil multifunctionality in the same agroecosystem.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14030594/s1, Table S1: Linear mixed-effects model of crop type, soil depth and their interactive effects on soil physical, chemical, and microbial properties; Table S2: Linear mixed-effects model of crop type, soil depth, and their interactive effects on soil extracellular enzymatic activities; Table S3: Linear mixed-effects model of crop type, soil depth, and their interactive effects on soil multifunctionality.

Author Contributions: Conceptualization, D.W. and J.C.; methodology, D.W. and J.C.; software, P.L.; validation, D.W. and J.C.; formal analysis, P.L.; investigation, P.L.; resources, D.W. and J.C.; data curation, P.L.; writing—original draft preparation, P.L.; writing—review and editing, D.W., J.C., Y.L., J.L., Y.C., G.L., C.W. (Chaoqun Wang), C.W. (Chao Wang) and D.L.M.; visualization, P.L.; supervision, D.W. and J.C.; project administration, D.W. and J.C.; funding acquisition, D.W. and J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by EU H2020 Marie Skłodowska-Curie Actions (No. 839806), Aarhus University Research Foundation (AUFF-E-2019-7-1), Danish Independent Research Foundation (1127-00015B), Nordic Committee of Agriculture and Food Research, Aarhus University iClimate, and Natural Science Basic Research Program of Shaanxi Program (No. 2022JQ-897), and the National Natural Science Foundation of China (NSFC32130066, 32371672).

Data Availability Statement: The datasets presented in this article are not readily available because the data are part of an ongoing study.

Acknowledgments: The contributions of all the researchers involved in this study are greatly appreciated.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Pimentel, D.; Cerasale, D.; Stanley, R.C.; Perlman, R.; Newman, E.M.; Brent, L.C.; Mullan, A.; Chang, D.T.I. Annual vs. perennial grain production. *Agric. Ecosyst. Environ.* **2012**, *161*, 1–9. [CrossRef]
2. Malik, A.A.; Puissant, J.; Buckeridge, K.M.; Goodall, T.; Jehmlich, N.; Chowdhury, S.; Gweon, H.S.; Peyton, J.M.; Mason, K.E.; van Agtmaal, M.; et al. Land use driven change in soil pH affects microbial carbon cycling processes. *Nat. Commun.* **2018**, *9*, 3591. [CrossRef] [PubMed]
3. Yang, C.; Geng, Y.; Fu, X.Z.; Coulter, J.A.; Chai, Q. The effects of wind erosion depending on cropping system and tillage method in a semi-arid region. *Agronomy* **2020**, *10*, 732. [CrossRef]
4. Singh, B.K.; Trivedi, P.; Egidi, E.; Macdonald, C.A.; Delgado-Baquerizo, M. Crop microbiome and sustainable agriculture. *Nat. Rev. Microbiol.* **2020**, *18*, 601–602. [CrossRef] [PubMed]
5. Li, X.; Wang, Z.; Bao, X.; Sun, J.; Yang, S.; Wang, P.; Wang, C.; Wu, J.; Liu, X.; Tian, X.; et al. Long-term increased grain yields and soil fertility from intercropping. *Nat. Sustain.* **2021**, *4*, 943–950. [CrossRef]
6. Tilman, D.; Balzer, C.; Hill, J.; Befort, B.L. Global food demand and the sustainable intensification of agriculture. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 20260–20264. [CrossRef]
7. Fan, K.; Chu, H.; Eldridge, D.J.; Gaitan, J.J.; Liu, Y.R.; Sokoya, B.; Wang, J.T.; Hu, H.W.; He, J.Z.; Sun, W.; et al. Soil biodiversity supports the delivery of multiple ecosystem functions in urban greenspaces. *Nat. Ecol. Evol.* **2023**, *7*, 113–126. [CrossRef]
8. Byrnes, J.E.K.; Gamfeldt, L.; Isbell, F.; Lefcheck, J.S.; Griffin, J.N.; Hector, A.; Cardinale, B.J.; Hooper, D.U.; Dee, L.E.; Emmett Duffy, J. Investigating the relationship between biodiversity and ecosystem multifunctionality: Challenges and solutions. *Methods Ecol. Evol.* **2014**, *5*, 111–124. [CrossRef]
9. Su, G.; Ong, H.C.; Zulkifli, N.W.M.; Ibrahim, S.; Chen, W.H.; Chong, C.T.; Ok, Y.S. Valorization of animal manure via pyrolysis for bioenergy: A review. *J. Clean. Prod.* **2022**, *343*, 130965. [CrossRef]
10. Cappelli, S.L.; Domeignoz-Horta, L.A.; Loaiza, V.; Laine, A.L. Plant biodiversity promotes sustainable agriculture directly and via belowground effects. *Trends Plant Sci.* **2022**, *27*, 674–687. [CrossRef]
11. Garland, G.; Edlinger, A.; Banerjee, S.; Degrune, F.; García-Palacios, P.; Pescador, D.S.; Herzog, C.; Romdhane, S.; Saghai, A.; Spor, A.; et al. Crop cover is more important than rotational diversity for soil multifunctionality and cereal yields in European cropping systems. *Nat. Food* **2021**, *2*, 28–37. [CrossRef]
12. Chen, J.; Manevski, K.; Lærke, P.E.; Jørgensen, U. Biomass yields, yields stability and soil carbon and nitrogen content under cropping systems destined for biorefineries. *Soil Tillage Res.* **2022**, *221*, 105397. [CrossRef]
13. Lanker, M.; Bell, M.; Picasso, V.D. Farmer perspectives and experiences introducing the novel perennial grain Kernza intermediate wheatgrass in the US Midwest. *Renew. Agric. Food Syst.* **2020**, *35*, 653–662. [CrossRef]
14. Qiao, Z.; Yates, T.B.; Shrestha, H.K.; Engle, N.L.; Flanagan, A.; Morrell-Falvey, J.L.; Sun, Y.; Tschaplinski, T.J.; Abraham, P.E.; Labbé, J.; et al. Towards engineering ectomycorrhization into switchgrass bioenergy crops via a lectin receptor-like kinase. *Plant Biotechnol. J.* **2021**, *19*, 2454–2468. [CrossRef] [PubMed]
15. Schipanski, M.E.; Barbercheck, M.; Douglas, M.R.; Finney, D.M.; Haider, K.; Kaye, J.P.; Kemanian, A.R.; Mortensen, D.A.; Ryan, M.R.; Tooker, J.; et al. A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agric. Syst.* **2014**, *125*, 12–22. [CrossRef]
16. Archer, N.A.L.; Quinton, J.N.; Hess, T.M. Below-ground relationships of soil texture, roots and hydraulic conductivity in two-phase mosaic vegetation in South-east Spain. *J. Arid Environ.* **2002**, *52*, 535–553. [CrossRef]
17. Halli, H.M.; Govindasamy, P.; Chaudhary, M.; Srinivasan, R.; Prasad, M.; Wasnik, V.K.; Yadav, V.K.; Singh, A.K.; Kumar, S.; Vijay, D.; et al. Range grasses to improve soil properties, carbon sustainability, and fodder security in degraded lands of semi-arid regions. *Sci. Total Environ.* **2022**, *851*, 158211. [CrossRef] [PubMed]
18. Zhang, J.; Feng, Y.; Maestre, F.T.; Berdugo, M.; Wang, J.; Coleine, C.; Saez-Sandino, T.; Garcia-Velazquez, L.; Singh, B.K.; Delgado-Baquerizo, M. Water availability creates global thresholds in multidimensional soil biodiversity and functions. *Nat. Ecol. Evol.* **2023**, *7*, 1002–1011. [CrossRef]
19. Chen, J.; Luo, Y.; Li, J.; Zhou, X.; Cao, J.; Wang, R.W.; Wang, Y.; Shelton, S.; Jin, Z.; Walker, L.M.; et al. Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Glob. Change Biol.* **2017**, *23*, 1328–1337. [CrossRef]

20. Huang, Z.; Sun, L.; Liu, Y.; Liu, Y.F.; López-Vicente, M.; Wei, X.H.; Wu, G.L. Alfalfa planting significantly improved alpine soil water infiltrability in the Qinghai-Tibetan Plateau. *Agric. Ecosyst. Environ.* **2019**, *285*, 106606. [CrossRef]
21. Li, L.; Li, S.M.; Sun, J.H.; Zhou, L.L.; Bao, X.G.; Zhang, H.G.; Zhang, F.S. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. *Proc. Natl. Acad. Sci USA* **2007**, *104*, 11192–11196. [CrossRef] [PubMed]
22. Cox, S.; Peel, M.D.; Creech, J.E.; Waldron, B.L.; Eun, J.; Zobell, D.R.; Miller, R.L.; Snyder, D.L. Forage production of grass–legume binary mixtures on Intermountain western USA irrigated pastures. *Crop Sci.* **2017**, *57*, 1742–1753. [CrossRef]
23. Zhang, J.; Dolfing, J.; Liu, W.; Chen, R.; Zhang, J.; Lin, X.; Feng, Y. Beyond the snapshot: Identification of the timeless, enduring indicator microbiome informing soil fertility and crop production in alkaline soils. *Environ. Microbiome* **2022**, *17*, 25. [CrossRef] [PubMed]
24. Chen, X.; Mo, X.; Hu, S.; Liu, S. Relationship between fluorescence yield and photochemical yield under water stress and intermediate light conditions. *J. Exp. Bot.* **2019**, *70*, 301–313. [CrossRef] [PubMed]
25. Ye, J.S.; Delgado-Baquerizo, M.; Soliveres, S.; Maestre, F.T. Multifunctionality debt in global drylands linked to past biome and climate. *Glob. Change Biol.* **2019**, *25*, 2152–2161. [CrossRef] [PubMed]
26. Wang, D.; Liu, C.; Yang, Y.; Liu, P.; Hu, W.; Song, H.; Miao, C.; Chen, J.; Yang, Z.; Miao, Y. Clipping decreases plant cover, litter mass, and water infiltration rate in soil across six plant community sites in a semiarid grassland. *Sci. Total Environ.* **2023**, *861*, 160692. [CrossRef]
27. Su, L.; Yang, Y.S.; Li, X.Y.; Wang, D.; Liu, Y.C.; Liu, Y.Z.; Yang, Z.L.; Li, M.M. Increasing plant diversity and forb ratio during the revegetation processes of trampled areas and trails enhances soil infiltration. *Land Degrad. Dev.* **2018**, *29*, 4025–4034. [CrossRef]
28. Huang, Z.; Liu, Y.F.; Cui, Z.; Liu, Y.; Wang, D.; Tian, F.P.; Wu, G.L. Natural grasslands maintain soil water sustainability better than planted grasslands in arid areas. *Agric. Ecosyst. Environ.* **2019**, *286*, 106683. [CrossRef]
29. Brookes, P.; Landman, A.; Pruden, G.; Jenkinson, D. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* **1985**, *17*, 837–842. [CrossRef]
30. DeForest, J.L. The influence of time, storage temperature, and substrate age on poten tial soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. *Soil Biol. Biochem.* **2009**, *41*, 1180–1186. [CrossRef]
31. Marx, M.-C.; Wood, M.; Jarvis, S.C. A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biol. Biochem.* **2001**, *33*, 1633–1640. [CrossRef]
32. García-Ruiz, R.; Ochoa, V.; Hinojosa, M.B.; Carreira, J.A. Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. *Soil Biol. Biochem.* **2008**, *40*, 2137–2145. [CrossRef]
33. Jia, R.; Zhou, J.; Chu, J.; Shahbaz, M.; Yang, Y.; Jones, D.L.; Zang, H.; Razavi, B.S.; Zeng, Z. Insights into the associations between soil quality and ecosystem multifunctionality driven by fertilization management: A case study from the North China plain. *J. Clean. Prod.* **2022**, *362*, 132265. [CrossRef]
34. Delgado-Baquerizo, M.; Maestre, F.T.; Reich, P.B.; Jeffries, T.C.; Gaitan, J.J.; Encinar, D.; Berdugo, M.; Campbell, C.D.; Singh, B.K. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nat. Commun.* **2016**, *7*, 10541. [CrossRef]
35. Schaller, J.; Puppe, D. Heat improves silicon availability in mineral soils. *Geoderma* **2021**, *386*, 114909. [CrossRef]
36. Shi, X.; Wang, J.; Lucas-Borja, M.E.; Wang, Z.; Li, X.; Huang, Z. Microbial diversity regulates ecosystem multifunctionality during natural secondary succession. *J. Appl. Ecol.* **2021**, *58*, 2833–2842. [CrossRef]
37. Pinheiro, J.; Bates, D.; DebRoy, S.; Sarkar, D.; R Core Team. nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-164. 2013. Available online: https://cran.r-project.org/web/packages/nlme/index.html (accessed on 1 November 2021).
38. Moon, K.-W. Make Interactive "ggplot2". Extension to "ggplot2" and "ggiraph" [R package ggiraphExtra version 0.3.0]. 2020. Available online: https://CRAN.R-project.org/package=ggiraphExtra (accessed on 15 November 2023).
39. Giling, D.P.; Beaumelle, L.; Phillips, H.R.P.; Cesarz, S.; Eisenhauer, N.; Ferlian, O.; Gottschall, F.; Guerra, C.; Hines, J.; Sendek, A.; et al. Aniche for ecosystem multifunctionality in global change research. *Glob. Chang. Biol.* **2019**, *25*, 763–774. [CrossRef]
40. Qiu, L.; Zhang, Q.; Zhu, H.; Reich, P.B.; Banerjee, S.; van der Heijden, M.G.A.; Sadowsky, M.J.; Ishii, S.; Jia, X.; Shao, M.; et al. Erosion reduces soil microbial diversity, network complexity and multifunctionality. *ISME J.* **2021**, *15*, 2474–2489. [CrossRef]
41. Perkins, D.M.; Bailey, R.A.; Dossena, M.; Gamfeldt, L.; Reiss, J.; Trimmer, M.; Woodward, G. Higher biodiversity is required to sustain multiple ecosystem processes across temperature regimes. *Glob. Change Biol.* **2015**, *21*, 396–406. [CrossRef]
42. Tian, D.; Xiang, Y.; Seabloom, E.; Chen, H.Y.H.; Wang, J.; Yu, G.; Deng, Y.; Li, Z.; Niu, S. Ecosystem restoration and belowground multifunctionality: A network view. *Ecol. Appl.* **2022**, *32*, e2575. [CrossRef]
43. Shen, H.J.; Zhang, Q.Q.; Zhang, X.; Jiang, X.Y.; Zhu, S.G.; Chen, A.F.; Wu, Z.; Xiong, Z.Q. In situ effects of biochar field-aged for six years on net N mineralization in paddy soil. *Soil Tillage Res.* **2021**, *205*, 104766. [CrossRef]
44. Benavent-González, A.; Delgado-Baquerizo, M.; Fernández-Brun, L.; Singh, B.K.; Maestre, F.T.; Sancho, L.G. Identity of plant, lichen and moss species connects with microbial abundance and soil functioning in maritime Antarctica. *Plant Soil* **2018**, *429*, 35–52. [CrossRef]
45. Zheng, J.; Zhang, F.; Zhang, B.; Chen, D.; Li, S.; Zhao, T.; Wang, Q.; Han, G.; Zhao, M. Biodiversity and soil pH regulate the recovery of ecosystem multifunctionality during secondary succession of abandoned croplands in northern China. *J. Environ. Manag.* **2023**, *327*, 116882. [CrossRef]
46. Albert, Á.; Kelemen, A.; Valkó, O.; Miglécz, T.; Csecserits, A.; Rédei, T.; Deák, B.; Tóthmérész, B.; Török, P. Secondary succession in sandy old fields: A promising example of spontaneous grassland recovery. *Appl. Veg. Sci.* **2014**, *17*, 214–224. [CrossRef]

47. Weigelt, A.; Jolliffe, P. Indices of plant competition. *J. Ecol.* **2003**, *91*, 707–720. [CrossRef]
48. Liu, Y.; Guo, L.; Huang, Z.; Lopez-Vicente, M.; Wu, G.L. Root morphological characteristics and soil water infiltration capacity in semi-arid artificial grassland soils. *Agric. Water Manag.* **2020**, *235*, 106153. [CrossRef]
49. Zhao, D.; Xu, M.; Liu, G.; Ma, L.; Zhang, S.; Xiao, T.; Peng, G. Effect of vegetation type on microstructure of soil aggregates on the Loess Plateau, China. *Agric. Ecosyst. Environ.* **2017**, *242*, 1–8. [CrossRef]
50. Delgado-Baquerizo, M.; Reich, P.B.; Trivedi, C.; Eldridge, D.J.; Abades, S.; Alfaro, F.D.; Bastida, F.; Berhe, A.A.; Cutler, N.A.; Gallardo, A.; et al. Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nat. Ecol. Evol.* **2020**, *4*, 210–220. [CrossRef]
51. Xu, R.; Shi, W.; Kamran, M.; Chang, S.; Jia, Q.; Hou, F. Grass-legume mixture and nitrogen application improve yield, quality, and water and nitrogen utilization efficiency of grazed pastures in the loess plateau. *Front. Plant Sci.* **2023**, *14*, 088849. [CrossRef] [PubMed]
52. Wittwer, R.A.; Bender, S.F.; Hartman, K.; Hydbom, S.; Lima, R.A.A.; Loaiza, V.; Nemecek, T.; Oehl, F.; Olsson, P.A.; Petchey, O.; et al. Organic and conservation agriculture promote ecosystem multifunctionality. *Sci. Adv.* **2021**, *7*, eabg6995. [CrossRef] [PubMed]