



Intercropping improves soil ecosystem multifunctionality through enhanced available nutrients but depends on regional factors

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Abstract

Purpose Intercropping is an important agricultural management that has been applied worldwide. Although intercropping improves soil nutrients and crop productivity, its effects on the microbial-mediated belowground processes and main drivers remain unclear.

Methods We performed the same field study at two sites (Site1, Youyu; Site2, Zhangbei) by growing soybean and oat in monoculture and intercropping to investigate their effects on rhizosphere soil properties,

enzyme stoichiometry, and soil ecosystem multifunctionality (EMF).

Results Intercropping increased available phosphorus (Avail-P) by 87% and 16% for oat and soybean compared to the corresponding monoculture in site1, respectively. We also found that intercropping increased the C-acquiring and N-acquiring enzyme activities by 18%–48% in site1. Moreover, intercropping enhanced soil EMF and alleviated microbial P limitations for both oat and soybean compared to the corresponding monoculture in site1. However, all observed parameters were not affected by intercropping in site2, which may be due to the lower Avail-P, mineral nitrogen (N_{\min}), and precipitation in site2 compared to site1. Moreover, the soil EMF was strongly positively correlated with soil N_{\min} , Avail-P, air temperature, and precipitation.

Conclusion Therefore, intercropping improves soil ecosystem multifunctionality by increasing available nutrients, which are regulated by regional factors.

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Introduction

Intercropping is an important sustainable agricultural practice to meet local food demand and ensure food security, in which two or more crops are grown

together in the same field (Willey 1979; Martin-Guay et al. 2018; Qian et al. 2018). Due to the optimized use of resources, yields are normally higher in intercropping than those in monoculture (Hong et al. 2017; Qian et al. 2018), specifically in legume/cereal systems. Several legume crops can improve soil nitrogen (N) availability via atmospheric leguminous N_2 fixation (Li et al. 2016; Zang et al. 2018; Wang et al. 2019), which benefits neighboring plants and coupled with the acquisition of phosphorus (P) for neighboring plants in response to protons released from legumes (Zhang et al. 2017; Liao et al. 2020). Although previous studies have documented that inter-specific facilitation and spatio-temporal resource partitioning are among the driving factors of yield advantage in intercropping systems (Liu et al. 2018; Rodriguez et al. 2020; Li et al. 2020), its effects on microbial-mediated nutrient cycling and subsequent ecosystem functions remain largely unknown.

The rhizosphere soil is a dynamic microbial hotspot (Zhang et al. 2020) and leads an essential role in enhancing microbial metabolic activity (Kuzya-kov et al. 2007; Cui et al. 2018; Zhou et al. 2021a). Exoenzymes secreted by microbes are one of the main mediators of soil biological processes and play a vital role in sustaining soil quality and crop health (Sinsabaugh et al. 2009; Wallenstein and Burns 2011). As various crops have a specific influence on microbial communities in the rhizosphere (Marschner et al. 2004), variations in a suite of enzymes in their rhizosphere are more likely to come when crops are grown together. Intercropping has been documented to increase the soil enzyme activities such as acid phosphatase activity (Gong et al. 2019), while others have reported no effect (Wang et al. 2015; Solanki et al. 2019). Further, the effect of intercropping on soil enzyme activities seems to be enzyme-specific (Kumar et al. 2021). These inconsistent results thus call for additional studies to enable a better understanding of the impact of intercropping on soil enzyme activities in the rhizosphere.

Enzymatic stoichiometry is a widely used indicator to represent the features of microbial metabolic limitations (Sinsabaugh et al. 2009) and reveals the relationships between microbial metabolic requirement and soil nutrient supply (Sinsabaugh and Follstad Shah 2012; Cui et al. 2019). The status of microbial resource limitations can be altered by intercropping, owing to the shift in the quantity and quality of root

exudates caused by increased plant biodiversity. Intercropping can have a significant role in belowground interactions with their roots having a selective effect on rhizosphere microorganisms (Li et al. 2016). As a consequence, intercropping effect on exoenzyme stoichiometry would reveal the change in soil microbial nutrient requirements with positive or negative consequences on microbial processes (Sinsabaugh et al. 2009). Understanding resource-limited growth and activity of microorganisms is therefore important for improving soil nutrient cycling and crop productivity.

Soil ecosystem multifunctionality (EMF; i.e., performing multiple functions simultaneously) is increasingly applied in agroecosystems to offer a quantitative way to assess the functional roles of soil biodiversity (Allan et al. 2015; Jing et al. 2015). Soil EMF supplies the foundation for a solid statistical method that causes the synthesis of numerous different functions that soil organisms supply (Delgado-Baquerizo et al. 2016). Fundamental to the soil EMF approach is knowledge of nutrient cycling and the enzymes that catalyze these reactions in soil (Nannipieri et al. 2012). Intercropping can enhance ecosystem services and support multifunctionality (Ryan et al. 2018), such as increasing the crop yield stability and soil health (Bybee-Finley et al. 2016), as well as reducing nonpoint source pollution by decreasing N losses (Sanderson et al. 2013). However, these studies evaluated a single function, and a lack of comprehensive evaluation of multiple soil EMF simultaneously may prevent an understanding of intercropping on multiple functions (Allan et al. 2015). Therefore, quantifying the performance of soil EMF is an urgent issue, which will help us understand the effect of intercropping on nutrient cycling and soil health.

In a recent meta-analysis, Curtright and Tiemann (2021) have documented that intercropping significantly increases enzyme activities by 13% in North America and Europe and it is enzyme-dependent. However, less is known about the intercropping effect in the semi-arid area of Northern China, which plays a crucial role in national food security with an intensive agriculture system. Here, a field study of oat/soybean intercropping was established in Youyu and repeated in Zhangbei county in Northern China. We aimed (a) to investigate the influences of intercropping on soil properties, enzyme activities, and soil EMF in two sites; (b) to estimate the microbial resource limitation between monoculture and intercropping systems; (c) to

determine the main drivers of soil EMF in response to intercropping.

Materials and methods

Study site description

The field experiment was conducted at Youyu county, Shanxi province (40°00' N, 112°45' E; 1345 m above sea level; Site1), and Zhangbei county, Hebei province (41°34' N, 114°91' E; 1352 m above sea level; Site2), China. These two sites are located in the semi-arid area of Northern China, which has a typical temperate continental monsoon climate with a cold winter and hot summer. In site1, the mean monthly air temperature and precipitation from May to September were 16.5 °C and 378.5 mm, respectively. In the top 20 cm soil layer, the initial soil properties were soil organic carbon (SOC) 5.9 g kg⁻¹, total nitrogen (TN) 0.67 g kg⁻¹, available P (Avail-P) 26.2 mg kg⁻¹, mineral nitrogen (N_{min}) 6.1 mg kg⁻¹, and pH (H₂O) 8.1. In site2, the mean monthly air temperature and precipitation from May to September were 16.2 °C and 296.0 mm, respectively. The initial soil properties of the top 20 cm layer were SOC 7.5 g kg⁻¹, TN 0.97 g kg⁻¹, Avail-P 5.0 mg kg⁻¹, N_{min} 2.0 mg kg⁻¹, and pH (H₂O) 8.0. The preceding crop of the two experiment fields was oat. The air temperature and precipitation during the crop growing season are presented in Figure S1.

Experimental design

Across the two sites, the same field experimental design was arranged as follows: (1) monoculture oat (*Avena sativa* L.); (2) monoculture soybean (*Glycine max* L.); and (3) oat intercropped with soybean. The plots were randomly arranged with four replicates in both sites. The plot size was 20 m² (5 m × 4 m). Based on local agronomic performance, the tested cultivars of oat and soybean were Bayou 14 and Jizhangdou 2, respectively. Oat monoculture was planted with a row spacing of 25 cm and at a seeding rate of 150 kg ha⁻¹. Soybean monoculture was planted with a row spacing of 40 cm, plant spacing of 15 cm, and a seeding rate of 75 kg ha⁻¹. For intercropping systems, oat and soybean were planted in alternating 4 m wide strips,

which included a 2 m wide oat strip of 8 rows and a 2 m soybean strip of 5 rows and 32.5 cm inner-row distance. In intercropping systems, the seeding rate of oat was 75 kg ha⁻¹, while the row and plant spacing of soybean were similar to monocultures. Oat and soybean were sown in early May and harvested in late September 2019.

Soil sample collection

Soils were sampled from the oat and soybean rhizosphere at maturity in September 2019. Briefly, 12 roots of oat and soybean were randomly selected and excavated at each plot. The soil tightly adhering to the root surface (thickness < 1–2 mm) was referred to as rhizosphere soil, which was brushed with a sterile soft-bristled paintbrush physically. The rhizosphere soils collected from each root for each cropping system were pooled to form a mixed soil sample. For the intercropping system, the oat and soybean from the border row were selected to estimate the interaction between plants. Each composite sample was put in an icebox and taken back to the laboratory and the soil was separated into two subsamples. One subsample was stored at 4 °C (within two weeks) for the analysis of enzyme activity, as well as the measurement of ammonium (NH₄⁺), nitrate (NO₃⁻), and Avail-P. The second subsample was passed through a 2-mm sieve and air-dried for additional chemical analysis.

Soil chemical analysis

Soil pH was measured with a glass electrode (FE20, Mettler-Toledo, USA), with a soil and water ratio of 1:2.5 (w/v). SOC and TN were analyzed via the K₂Cr₂O₇ oxidation–reduction titration method and the Kjeldahl method, respectively (Bao 2000). N_{min} (NH₄⁺ and NO₃⁻) was measured in the microplate spectrophotometer (Thermo1510, MULTISKAN GO, USA) with 2 M L⁻¹ KCl with a 1:5 ratio based on absorbance measurement (Miranda et al. 2001). Avail-P (Phosphate) was extracted from fresh soil (1 g) with 5 ml of 0.5 M NaHCO₃ (pH 8.5), then the extracts of the samples and standards were measured by the malachite green colorimetric method (Ohno and Zibilske 1991).

Assays of enzyme activities

The potential enzyme activities were determined by the methods described by Zhou et al. (2021b) and Jia et al. (2022) for three C-acquiring exoenzymes (β -cellobiohydrolase (CE), β -xylosidase (BX), and β -glucosidase (BG)), two N-acquiring exoenzymes (β -1,4-N-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP)), and one P-acquiring exoenzyme (acid phosphatase (AP)). The enzyme activities were measured by two synthetic fluorescent indicators: 7-amino-4-methylcoumarin (AMC) and 4-methylumbelliferone (MUF). Briefly, fresh soil of 1 g was homogenized in 50 mL distilled water on a constant-temperature shaker for 30 min. The soil suspension of 50 μ L was added into 96-well microplates while stirring the suspension to ensure uniformity. Subsequently, 50 μ L of buffer (0.1 M L⁻¹ MES or 0.05 M L⁻¹ TRIZMA) and substrate solution (100 μ L, 400 μ mol L⁻¹) were added. The microplates were measured at 60 and 120 min after substrate addition fluorometrically at an excitation wavelength of 355 nm and an emission wavelength of 450 nm (Thermo1510, MULTISKAN Sky, USA). Here, the fluorescence data (μ M) was converted to enzyme activity which is shown as nmol g⁻¹ h⁻¹, based on the standard curve for MUF and AMC standards.

Quantification of soil ecosystem multifunctionality and microbial metabolic limitation

Here, we used averaged mean extracellular enzyme activities (EEAs) as proxies for specific substrate or nutrient acquisition (Luo et al. 2018; Jia et al. 2022) including carbon (C-acquiring), nitrogen (N-acquiring), and phosphorus (P-acquiring) acquisition. The EEAs were calculated as follows:

$$C - acq = (CE + BX + BG)/3 \quad (1)$$

$$N - acq = (NAG + LAP)/2 \quad (2)$$

$$P - acq = AP \quad (3)$$

To acquire a quantitative soil multifunctionality index, we first normalized each of the eleven soil functions measured (C cycling: BG, BX, CE, and SOC; N cycling: NAG, LAP, TN, NH₄⁺-N,

and NO₃⁻-N; P cycling: AP and Avail-P) using the Z-score transformation (Delgado-Baquerizo et al. 2016; Jia et al. 2022). These standardized ecosystem functions were then averaged to obtain a soil multifunctionality index (Wagg et al. 2014).

The microbial metabolic limitation was determined by calculating the vector lengths and angles of enzymes according to untransformed proportional activities (e.g. [CE + BX + BG]/[CE + BX + BG + NAG + LAP]) (Moorhead et al. 2016). Vector lengths and angles were determined as the square root of the sum of x² and y² and the arctangent of the line extending from the plot origin to point (x, y) as shown by Eq. (4) and Eq. (5).

$$Vector\ length = \sqrt{x^2 + y^2} \quad (4)$$

$$Vector\ angle = DEGREES(ATAN2(x, y)) \quad (5)$$

where x and y represent the relative activity of C-versus P-acquiring enzymes and the relative activity of C-versus N-acquiring enzymes, respectively. Microbial C limitation increases with vector lengths. Vector angles > 45° indicate microbial P limitation, and microbial P limitation increases with vector angle. Vector angles < 45° represent N limitation and microbial N limitation decreases with vector angle.

Statistical analysis

The two-way ANOVA was applied to identify the effects of the crop (oat and soybean), pattern (monoculture and intercropping), and their interactions on soil physicochemical characteristics, enzyme activities, and microbial metabolic limitations. Significant differences were considered at *P* < 0.05. The generalized linear model was adopted to determine the relationships between microbial metabolic limitations and soil nutrients. The enzyme activities and environmental variables of the two experimental sites were analyzed separately based on principal component analysis (PCA). Correlations between soil EMF and environmental variables were performed by Mantel tests. The soil biochemical properties, enzyme activities, microbial metabolic limitations, the generalized linear model and PCA were analyzed for two sites separately, while the Mantel test was done based on the results from two sites together. Statistical analyses were carried out using SPSS v20.0 (IBM Inc.,

Armonk, USA). Sigmaplot v12.5 (Systat Software Inc., San Jose, CA, USA), OriginPro 2021 (Origin-Lab Corporation, Northampton, MA, USA), and R (version 4.1.2) were used for figure configuration.

Results

Soil properties and enzyme activities

In general, rhizosphere soil properties were mainly affected by crop species rather than intercropping (Fig. 1). For example, the mean pH in the soybean rhizosphere was significantly lower than oat regardless of experimental sites ($P < 0.05$, Fig. 1d). In contrast, the N_{\min} in the soybean rhizosphere was 1.6 and 3.0 times higher than in the oat rhizosphere in monoculture ($P < 0.05$, Fig. 1e). Remarkably, intercropping increased Avail-P by 87% and 16% for oat and soybean compared to the corresponding monoculture in site1 ($P < 0.05$). However, Avail-P was more affected by crops rather than intercropping in site2 (Fig. 1f). Furthermore, intercropping increased TN for oat whilst it decreased the TN for soybean relative to the corresponding monoculture in site1 (Fig. 1b). Overall, intercropping mainly changes Avail-P which was site-dependent, while the other rhizosphere soil properties were mainly controlled by crop type.

The effects of intercropping patterns on enzyme activities were dependent on experimental sites (Fig. 2). For example, intercropping increased C-acquiring enzyme activities by 47% and 18% for oat and soybean compared to the corresponding monoculture in site1 ($P < 0.05$; Fig. 2a). Similarly, the N-acquiring enzyme activities under intercropped oat and soybean in site1 were 20% and 36% higher than under the corresponding monoculture ($P < 0.05$; Fig. 2b). However, intercropping decreased the P-acquisition enzyme activity by 16% for oat whereas increased by 11% for soybean compared to the corresponding monoculture in site1 ($P < 0.05$; Fig. 2c). Additionally, C- and N-acquiring enzymes in site1 were higher under soybean compared with oat soils ($P < 0.05$). Conversely, the P-acquiring enzyme was lower in soybean than in oat. In site2, however, intercropping and crop did not change C-, N-, and P-acquiring enzymes compared to monoculture. Collectively, intercropping increased C- and N-acquiring enzymes but was site- and crop-specific.

Extracellular enzyme stoichiometry and ecosystem multifunctionality

All enzymatic stoichiometric points were above the 1:1 line, indicating microbial P limitations across both sites (Fig. 3a). In site1, compared to monoculture oat, the vector lengths (i.e. C limitation) displayed an increasing trend for intercropped oat, whereas no changes were detected between monoculture and intercropped soybean (Fig. 3b). Although vector angles from all treatments were larger than 45° , intercropping decreased vector angles compared to monoculture for both oat and soybean in site1 (Fig. 3c), indicating alleviated P limitation in soil. However, both vector lengths and angles were not affected by crops and the cropping system on site2 ($P > 0.05$). Additionally, the linear regression analysis identified a significant negative correlation between vector lengths and angles ($P < 0.05$; Fig. 3d) in site1 rather than site2. Specifically, the negative correlation between vector length and vector angle indicates a limitation of microbial metabolism in site1. Overall, this would suggest that intercropping could alleviate the P limitation but is dependent on experimental sites.

The result showed that the soil EMF was significantly affected by the interaction of crops and cropping patterns at site1 ($P < 0.05$; Fig. 4). For instance, intercropping increased soil EMF for oat compared to corresponding monoculture (0.45 vs. -0.25; $P < 0.05$), whilst no difference was observed for soybean ($P > 0.05$). In site2, however, soil EMF was lower than zero, and there was no difference between crops and intercropping patterns. Therefore, intercropping increased soil EMF which was regulated by regional factors and crop type.

The relationships between soil properties, enzyme activities, and soil ecosystem multifunctionality

The two main principal components (PC) 1 and 2 were selected with an explanation of 34% and 18% for site1, and 34% and 27% for site2, respectively (Fig. 5). The results of the principal component analysis (PCA) showed the effects of intercropping patterns on rhizosphere soil properties were dependent on experimental sites (Fig. 5). In site1, monoculture oat was positively correlated with AP and pH, while monoculture soybean was positively correlated with TN, SOC, and C:N ratio.

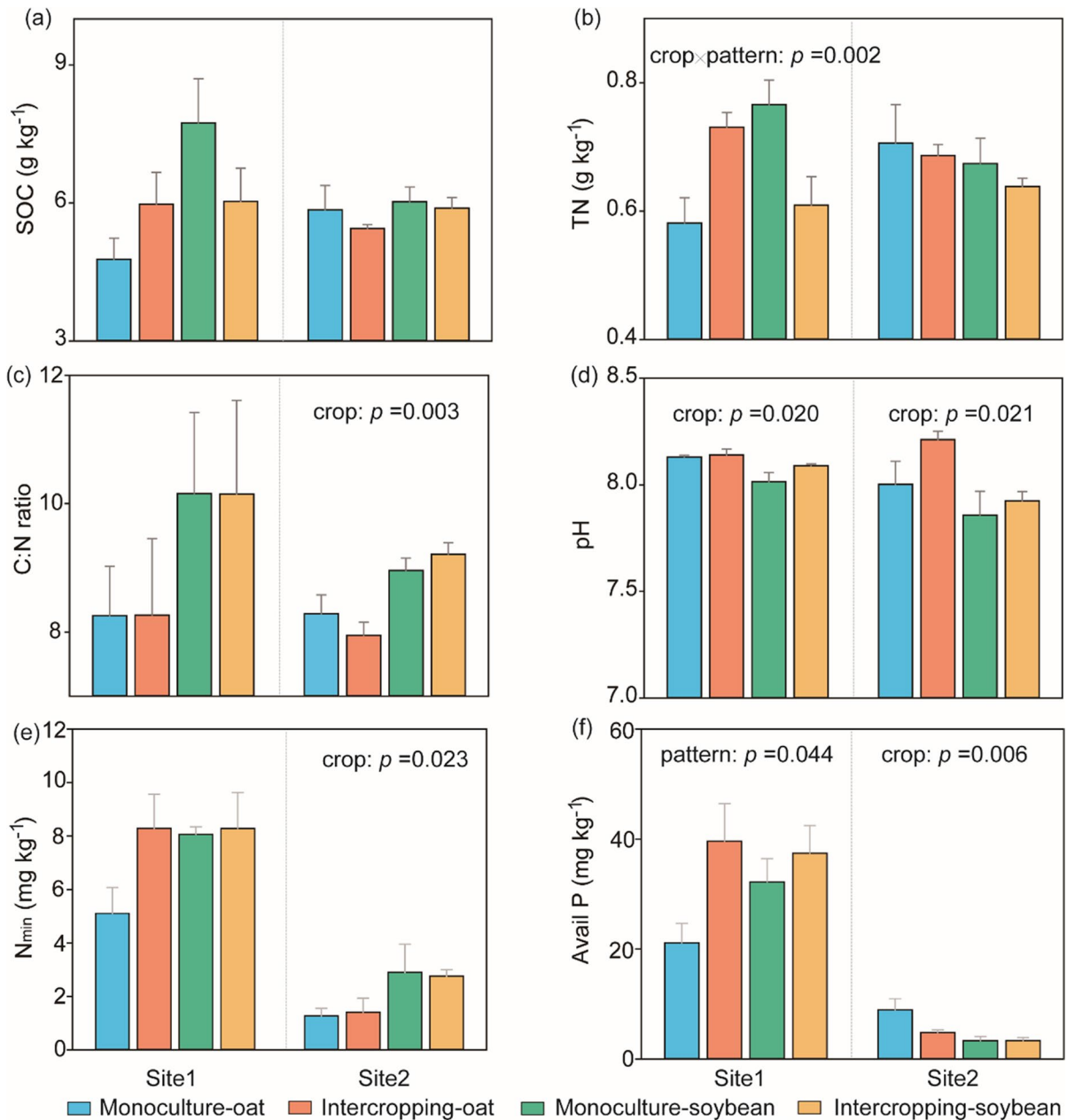


Fig. 1 Soil organic carbon (SOC) (a), total nitrogen (TN) (b), the ratio of SOC and TN (C:N ratio) (c), pH (d), mineral nitrogen (N_{\min}) (e), and available phosphorus (Avail-P) (f) in rhizosphere under oat and soybean in monoculture and intercropping system. Data represents means \pm SE ($n=4$). The P -value indicates significance based on a two-way ANOVA of crop and pattern. Note differences in the y-axis scale

ping system. Data represents means \pm SE ($n=4$). The P -value indicates significance based on a two-way ANOVA of crop and pattern. Note differences in the y-axis scale

Additionally, both intercropping oat and soybean were correlated with Avail-P, N_{\min} , CE, BG, BX, NAG, and LAP. In site2, both monoculture and intercropping oat

were positively correlated with pH, Avail-P, AP, BX, BG, LAP, and CE, while both monoculture and intercropping soybean were positively correlated with SOC,

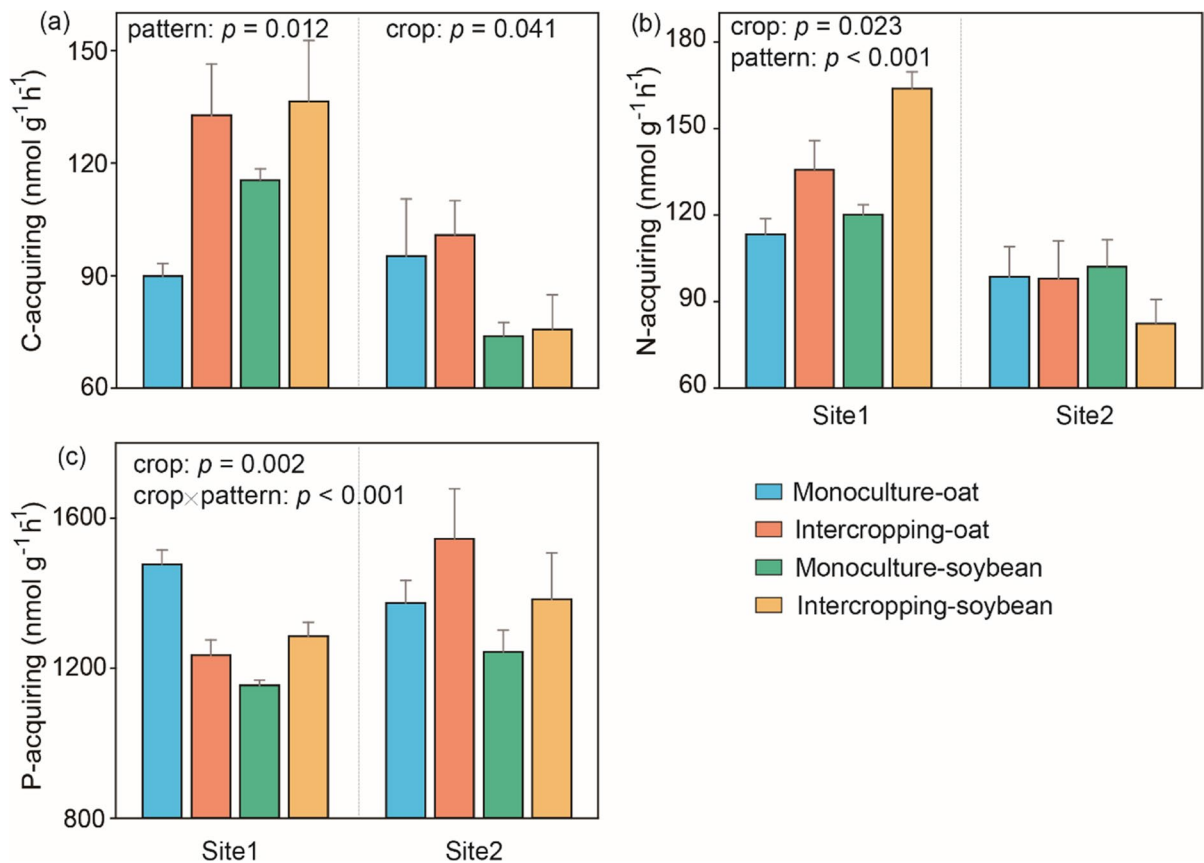


Fig. 2 Soil C- (a), N- (b), and P-acquiring (c) enzyme activities in rhizosphere under oat and soybean in monoculture and intercropping system. The C-acquiring enzyme activities represents the mean of β -Cellulohydrolase, β -xylosidase, and β -glucosidase. The N-acquiring enzyme activities represents

the mean of β -1,4-N-acetylglucosaminidase and L-leucine aminopeptidase. The P-acquiring enzyme activities represents acid phosphatase. Data represent means \pm SE ($n=4$). The P -value indicates significance based on a two-way ANOVA of crop and pattern

N_{\min} , and C:N ratio (Fig. 5b). Therefore, intercropping patterns changed the rhizosphere soil properties and enzyme activities, but the effect was site-dependent. The Mantel test analysis (correlation across two sites) revealed that soil EMF was strongly associated with most environmental factors (Fig. 6). The soil EMF was strongly positively correlated with rhizosphere soil N_{\min} (mantel's $r > 0.3$; $P < 0.01$), Avail-P (mantel's $r > 0.3$; $P < 0.01$), air temperature (mantel's $r < 0.3$; $P < 0.01$), and precipitation (mantel's $r < 0.3$; $P < 0.01$) across two sites. This indicates that changes in soil EMF were mainly driven by available nutrients in the rhizosphere.

Discussion

Soil properties and nutrients under intercropping

Intercropping increased soil Avail-P in the rhizosphere for both oat and soybean (Fig. 1f), which could be explained by the stimulated microbial P mineralization due to niche complementarity and interspecific facilitation (Xue et al. 2016). Root nodules of soybean could supply a large quantity of N (Li et al. 2016), decreasing the competition for nutrients and thus improving the nutrient accumulation in the oat

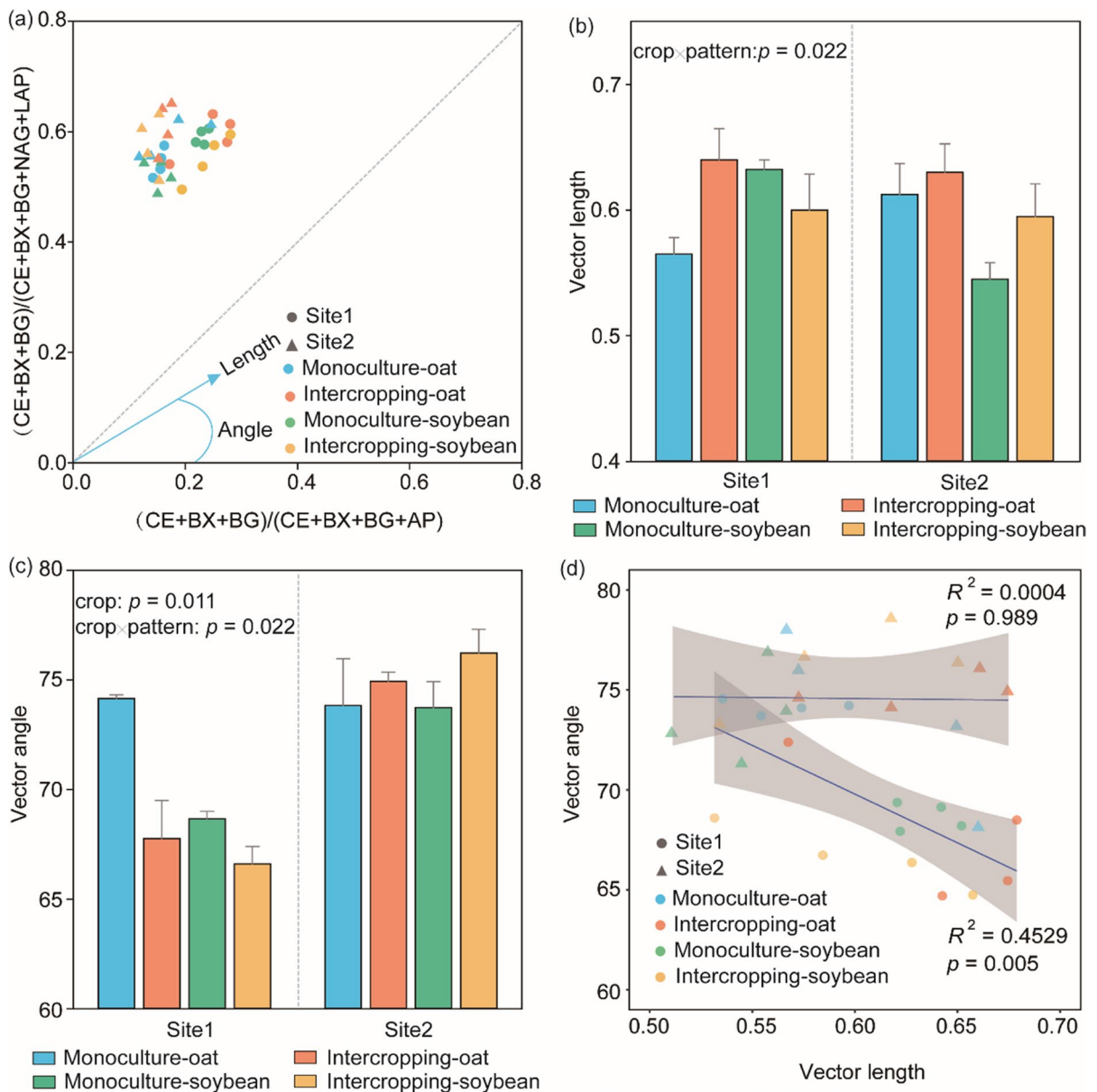


Fig. 3 Soil extracellular enzyme stoichiometry (a), vector length (b), vector angle (c), the relationship between vector length and angle (d) under oat and soybean in monoculture and intercropping system. β -Cellulohydrolase (CE); β -xylosidase (BX); β -glucosidase (BG); β -1,4-N-acetylglucosaminidase (NAG); L-leucine aminopeptidase (LAP); acid phosphatase (AP). Vector length represents soil C limitation for microbes and vector angle represents soil N or P limitation for microbes.

rhizosphere. Thereby, a higher amount of available N was found in the intercropped oat compared with the corresponding monoculture. Given that intercropped

Linear regression analysis was used to identify the relationships between microbial C limitation with microbial N or P limitation based on the relation between vector length and vector angle. The variation of vector length and vector angle, data represents means \pm SE ($n=4$). The P -value indicates significance based on two-way ANOVA of crop and pattern. Solid lines in d indicate the model fits between the vector length and vector angle, and the grey area is the 95% confidence interval

oat could uptake N from soybean N_2 fixation, lower TN and N_{min} were observed in the intercropped soybean. Furthermore, at the late stage, oat becomes

Fig. 4 Soil ecosystem multifunctionality under oat and soybean in monoculture and intercropping system. Data represents means \pm SE ($n=4$). The P -value indicates significance based on two-way ANOVA of crop and pattern

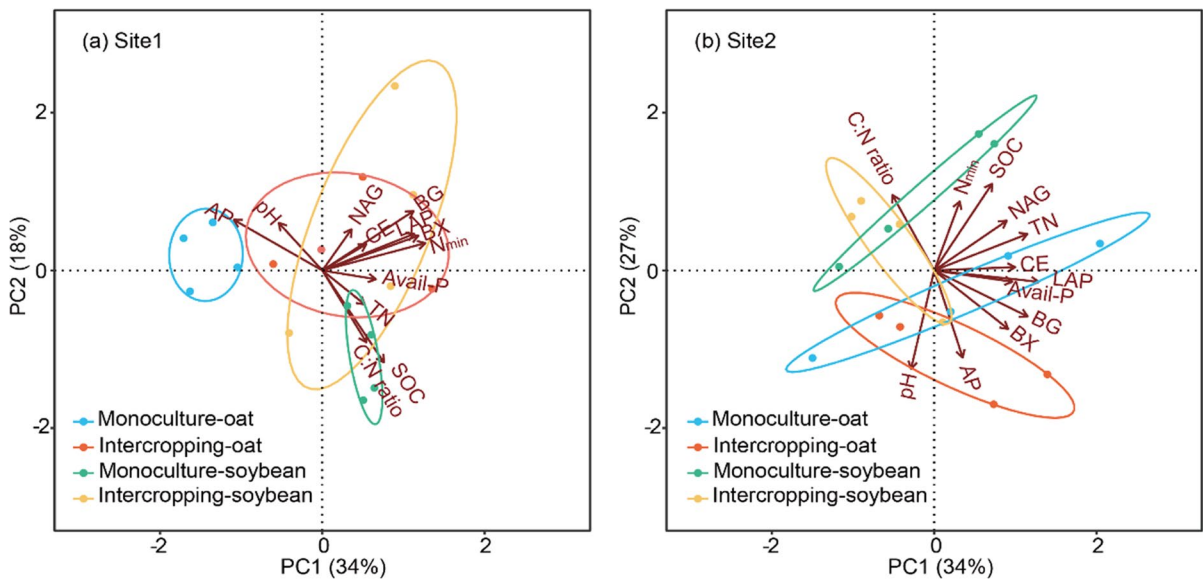
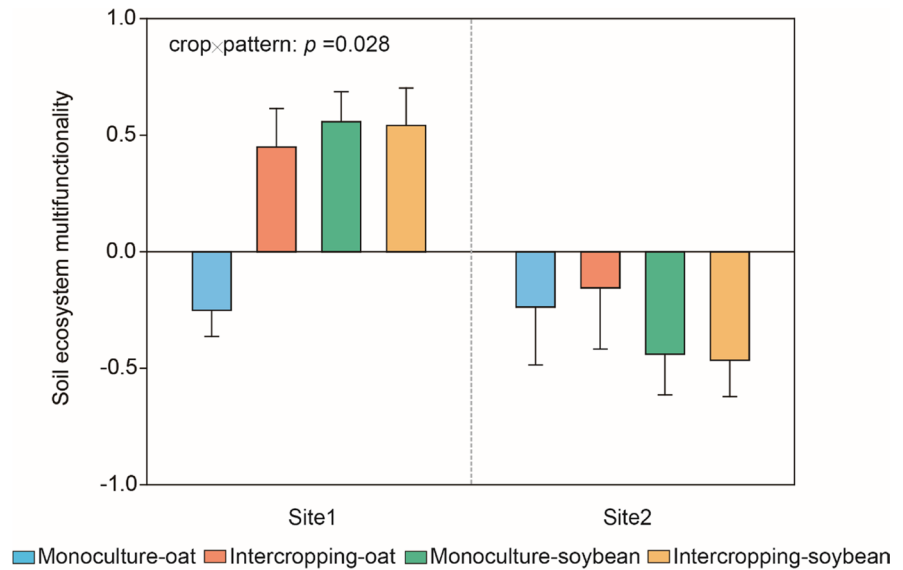


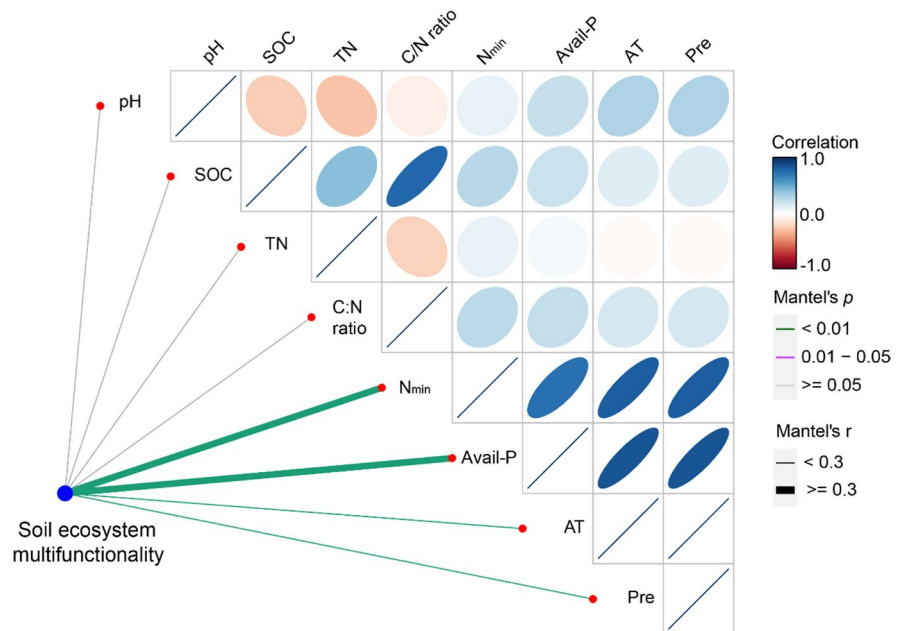
Fig. 5 The principal component analysis (PCA) between extracellular enzyme activities and soil physicochemical properties in rhizosphere under oat and soybean in monoculture and intercropping system. Soil organic carbon (SOC), total nitrogen (TN), SOC and TN ratio (C:N ratio), available phosphorus (Avail-P), mineral nitrogen (N_{min}), β -Cellobiohydrolase (CE), β -xylosidase (BX), β -glucosidase (BG), β -1,4-N-acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP), and acid phosphatase (AP)

phorus (Avail-P), mineral nitrogen (N_{min}), β -Cellobiohydrolase (CE), β -xylosidase (BX), β -glucosidase (BG), β -1,4-N-acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP), and acid phosphatase (AP)

taller than soybean in the intercropping system and has a greater capture of light (Tsay et al. 1988). This, in turn, resulted in stronger competition between crops and consequently might lower N rhizodeposition in the soybean rhizosphere. Further, intercropping increased TN for oat whilst it decreased that for

soybean relative to the corresponding monoculture, which may be due to niche complementarity and interspecific facilitation (Xue et al. 2016). In addition, our results showed that rhizosphere soil nutrients were mainly regulated by crop species (Fig. 1), a likely response to the variation in quality and quantity

Fig. 6 Relationships between environmental variables and soil ecosystem multifunctionality under oat and soybean in monoculture and intercropping system. SOC: soil organic carbon; TN: total nitrogen; C:N ratio: soil organic carbon, total nitrogen ratio; N_{\min} : mineral nitrogen (the sum of NH_4^+ + NO_3^-); Avail-P: available phosphorus; AT: air temperature; Pre: precipitation



of rhizosphere exudates. Collectively, intercropping can increase rhizosphere soil Avail-P and has a contrasting effect on N for oat and soybean, while other rhizosphere soil nutrients are mainly controlled by crop type.

Enzyme activities and stoichiometries under intercropping

Rhizosphere soil enzyme activities (C- and N-acquiring) of intercropped oat and soybean both increased in site1 (Fig. 2a). Three potential reasons can explain such a phenomenon. Firstly, compared with monoculture, intercropping increases total C input and diversity of compounds (i.e. glucose, cellulose, and proteins) into the soil (Curtright and Tiemann 2021), which may stimulate enzyme activities. Secondly, the higher N availability induced by legumes will also serve as N and energy sources for microbial growth and subsequently increase microbial activities (Sinsabaugh et al. 2008; Yan et al. 2022). Thirdly, enzymes are N-rich molecules and thus involve a high N cost for microbes making their products highly dependent on N in addition to C for energy (Frankena et al. 1988; Allison et al. 2010). This was also indicated by the positive correlation between enzyme activities and available nutrient contents (Fig. 5a), suggesting that enzyme production was mediated by resource

stoichiometries. In short, these results suggest that intercropping, as a form of spatial species diversity, can improve soil enzyme activities with consequences for ecosystem functioning.

Given the importance of type and quality of residue (Tiemann and Billings 2011), it is not surprising that the magnitude of enzyme responses to intercropping is sensitive to crop types. There was a tendency of larger C- and N-acquiring enzymes in the legume rhizosphere soil compared with oat, a potential result from a higher N input from N_2 fixation and then high-quality residues (narrow-C:N) production. It has previously been reported that legumes appear to induce stronger effects on enzyme activities compared with other crop types (Curtright and Tiemann 2021).

The soil enzymatic stoichiometry scatter plots showed soil microbes were P limited both in site1 and site2 (Fig. 3a), and intercropping seems to decrease the P limitation compared with monoculture in site1. This is also in line with legumes in the intercropping systems having an increased soil Avail-P content. However, the microbial communities were C and N limited under intercropping in the oat rhizosphere soil in site1, which was confirmed by the increased C and N-acquisition enzyme activities. As more enzymes are produced, more C as an energy source is required, which can further increase C limitations (Dodor et al. 2018). Further, intercropping with soybean improved

N availability, such as TN and N_{\min} in the oat rhizosphere soil, which also enhanced the demand for C and energy in microbial N metabolism. This can also be supported by the positive correlation between C limitation and soil N fractions (Fig. S2). Furthermore, intercropping did not alter enzyme stoichiometric ratios in site2, which may be due to the response to the homeostatic regulation of microorganisms. Therefore, intercropping can increase soil enzyme activities and alleviate microbial P limitations, but the microbial resources or microbial resource acquisition were site-specific.

Factors mediating soil EMF under intercropping systems

Intercropping promoted the soil EMF in site1, driven mainly by Avail-P and N_{\min} (Fig. 6). This indicates that higher nutrient availability favors microbial growth, ultimately accelerates biochemical cycles and improves ecological functions (Geyer et al. 2016; Han et al. 2021). Greater substrate availability in the intercropping systems is demonstrated by higher C- and N-acquiring enzyme activity. In addition, N benefits within intercropped crops will lead to increased C inputs to soil, stimulate the microbial community, and further improve soil biochemistry processes and EMF (Hong et al. 2017; Qian et al. 2018). However, the soil EMF in site2 was not influenced by intercropping (Fig. 5b), a response potentially related to nutrient deficiency and precipitation. This was supported by the positive correlation between soil EMF and available nutrients and precipitation (Fig. 6 and Fig. S3; Brooker et al. 2015; Curtright and Tiemann 2021). A meta-analysis found that the response of soil enzymes to intercropping depends largely on the sand fraction and available N content (Curtright and Tiemann 2021). There are higher available nutrients and lower sand fractions in site1 than site2 (Fig. 1e and f) and this was also shown by the positive correlation between enzyme activities and available nutrients (Fig. 5a). Furthermore, the initial TN content in site2 (0.97 g kg^{-1}) was higher than site1 (0.67 g kg^{-1}), while the initial N_{\min} content in site2 (2.0 mg kg^{-1}) was lower than site1 (6.1 mg kg^{-1}). In addition, there are higher sand fractions and lower precipitation in site2 than site1. Thus, it may be due to the high sand

fraction, which increases water leaching and resulted in lower N_{\min} content. This could in turn affects soil enzyme activities. These results document that the effect of intercropping depends on the initial soil nutrient status, sand content, and precipitation. To clarify the underlying microbial regulatory mechanisms, long-term in situ experiments are thus encouraged to better elucidate the role of intercropping on soil enzymes and nutrient cycles concerning the microbial community composition and functional gens.

Conclusions

In this study, intercropping increased Avail-P by 60% and 17% for oat and soybean compared to the corresponding monoculture in site1, respectively. Further, intercropping increased the C- and N-acquiring enzyme activities by 18–47% and 20–36% than the corresponding monoculture in site1, respectively. In addition, intercropping relieved the soil microbial P limitation and promoted soil ecosystem multifunctionality in site1. However, a strong influence of intercropping on those soil parameters in site2 was not evident, which is partly due to the lower Avail-P, N_{\min} , and precipitation. Overall, intercropping is a better practice in agroecosystems to improve soil ecosystem multifunctionality through enhanced available nutrients but depends on regional factors.

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