Maize-alfalfa intercropping promote ecosystem services than fertilized single crops

Tao, D.X.*; Gao, Y. Z.*
*Institute of Grassland Science, Key Laboratory of Vegetation Ecology of the Ministry of Education, Northeast Normal University, Changchun 130024, China; *Key Laboratory of Grassland Resources and Ecology of Western Arid Desert Area of the Ministry of Education, College of Grassland Science, Xinjiang Agricultural University, Urumqi 830052, China

Keywords: Multiple ecosystem services; microbial diversity; productivity; trade-offs; phosphorus.

Abstract. Phosphorus is a non-renewable source of fertilization, which will challenge the future of food production and cropland sustainability worldwide. Crop diversity is known to promote food production, yet its capacity to alleviate the dependence of multiple ecosystem services on non-renewable fertilization remains virtually unknown. Here, we conducted a field experiment to quantify the contribution of maize-alfalfa intercropping to support multiple ecosystem services under contrasting levels of phosphorus fertilization. We showed that unfertilized intercropping systems can support larger levels of multiple ecosystem services such as soil microbial habitat, plant-soil mutualism, nutrient cycling, and soil carbon storage compared with phosphorus-fertilized single crops. Intercropping also helped to reduce important tradeoffs in productivity and soil biodiversity compared with fertilized single crops. Together, our results provide evidence that intercropping systems are efficient in maintaining multiple ecosystem services and can help alleviate our global dependence on non-renewable fertilization.

Introduction

A growing human population and the demand for greater quality food is challenging the food production system worldwide. Soil nutrient depletion due to impacts of climate change and soil erosion, and food production is largely dependent on fertilization. Phosphorus is the most important non-renewable source of fertilization in croplands globally, which will challenge the future of food production and cropland sustainability worldwide. A solution for this fundamental problem may be crop diversity. Intercropping is known to promote food production, yet its capacity to alleviate the dependence of multiple ecosystem services on non-renewable fertilization remains virtually unknown.

Herein, we investigated how phosphorus fertilization and cropping patterns individually and interactively affected the multiple services in a 5-year experiment in an agriculture-pasturage ecotone in northeast China - one of the major global users of phosphorus fertilization. We used a maize (*Zea mays* L.) -alfalfa (*Medicago sativa* L.) intercropping system as our model system. Our goal is to evaluate whether intercropping can help alleviate the dependence of the multiple ecosystem services provided by croplands on non-renewal phosphorus fertilization, and further assess whether intercropping can reduce important ecosystem service trade-offs typically reported in croplands (e.g., productivity vs. soil biodiversity).

Methods and Study Site

The field experiment was conducted at Jilin Songnen Grassland Ecosystem National Observation and Research Station of the Northeast Normal University, Changling County, Jilin Province, Northeast China (123° 449′ E and 44° 409′ N, 137.8-144.8 m above sea level). The field experiment was established in 2014 and conducted with maize (*Zea mays* L. cv. Zhengdan 958) and alfalfa (*Medicago sativa* L. cv. Dongmu No. 1) in a 2 factorial completely randomized block design. Factors were composed of two phosphorus levels and three cropping patterns, with four replicates. Two phosphorus levels included with and without phosphorus (P) fertilization. Three cropping patterns include monoculture maize (MM), monoculture alfalfa (MA), maize and alfalfa intercropping (IMA). The plant and soil samples were collected in early October in 2017 and 2018. We determined the diversity of the soil bacterial and fungal communities using PCR amplicon sequencing. Equal molar amounts of the PCR products with barcodes were mixed and multiplexed sequenced using the Illumina MiSeq PE2500 platform (Illumina Inc., San Diego, USA). We assessed the performance of the different systems and determined the multiple ecosystem services using different approaches. We first used the averaging method by averaging these standardized ecosystem services to calculate the multiple ecosystem services. In addition, we performed a multiple threshold analysis on ecosystem services over a wide range of thresholds (25%, 50%, and 75%). Finally, the multi-dimension of ecosystem services was determined using principal component analysis.

To assess the overall performance of the investigated cropping systems, we calculated different multiple ecosystem services using 17 variables and 10 services, including microbial diversity (i.e., Shannon index of bacteria and fungi), productivity (yield), product quality (phosphorus concentration), soil microbial habitat (i.e., microbial biomass carbon, bacteria and fungi PLFAs), plant-soil mutualism (i.e., arbuscular mycorrhizal fungi (AMF) PLFAs, AMF and ectomycorrhizal fungi (EMF) relative abundance, plant pathogen (pathogen), nutrient cycling (i.e., total nitrogen, and available phosphorus), soil carbon storage (soil organic carbon), phosphorus utilization efficiency (phosphorus utilization efficiency), system stability and sustainability (i.e., coefficient of variation, and sustainable yield index) (Table S1). Before analyses, all individual ecosystem services (ES) variables were standardized by transformation as follows: ES=[rawES−min(rawES)]/[max(rawES)−min(rawES)], with ES indicating the final (transformed) ecosystem services value and raw ES indicating raw (untransformed) ecosystem services values. This way each transformed ES variable had a minimum value of zero and a maximum of one (Wang et al., 2019a).

The analyses of variances for microbial diversity, productivity, product quality, soil microbial habitat, plant-soil mutualism, plant pathogen, nutrient cycling, soil carbon storage, phosphorus utilization efficiency, system stability and sustainability, ecosystem services, and weighted ecosystem services were performed with mixed linear model in SPSS 23. Phosphorus level (P), cropping pattern (CP), and their interactions as fixed factors, and block and year as random factors to account for the repeated measures across 2017-2018. Variation Partitioning Modeling were used to quantify the relative importance of intercropping, phosphorus, and years on concerned ecosystem services. Specifically, this analysis allowed us to identify the unique and shared portion of the variation in the multiple ecosystem services explained by the three factors (Legendre, 2007). We used the varpart function from the “vegan” R package to run these analyses. To visualize the trade-off in ecosystem services, a correlations matrix was constructed using the R package “corrplot”. Correlation coefficients and significance ($p < 0.05$) were presented as a heat map with color legend representing positive and negative correlations and ellipses size illustrating the magnitude of correlation coefficient values.

Results and Discussion

Intercropping alleviates the dependence of multiple ecosystem services on non-renewal fertilization

Unfertilized intercropping promoted multiple ecosystem services compared with fertilized single crops (Fig. 1). In particular, unfertilized intercropping significantly increased the levels of soil microbial habitat, plant-soil mutualism, nutrient cycling, and soil carbon storage compared with phosphorus-fertilized single crops. (Fig. 2D, and F-H). Phosphorus addition significantly increased productivity, product quality, and nutrient cycling services than without phosphorus (Fig. 2A-B, and G). However, phosphorus addition decreased microbial diversity and plant-soil mutualism in 2017, and decreased soil microbial habitat and pathogen control in 2018 (Fig. 2C-F). Intercropping increased system stability and sustainability with phosphorus (Fig. 2J). More interestingly, the positive effect of phosphorus additions is very limited when using an intercropping approach. For example, phosphorus addition had no remarkable effect on plant-soil mutualism and soil carbon storage services in intercropping, while significantly decreased phosphorus utilization efficiency (Fig. 2D, H, and I).

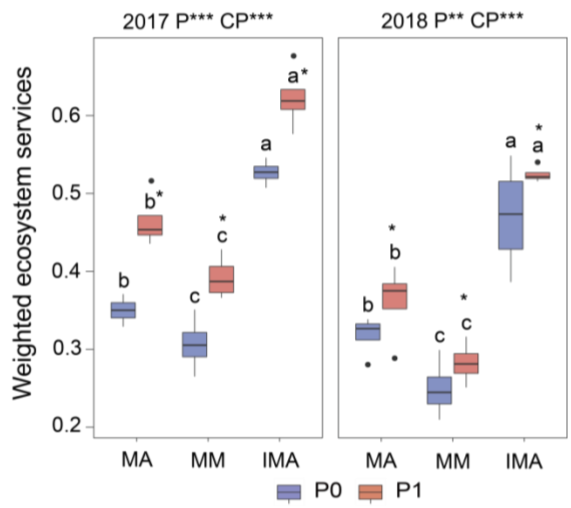

Figure1 Effects of phosphorus levels (P, P1: phosphorus addition, P0: without phosphorus), crop patterns (CP, MA: monoculture alfalfa, MM: monoculture maize, IMA: intercropping), and their interactions on weighted ecosystem services. The analyses of variances were used to test the significance of P and CP. For clarity, only the significant statistical results (*p* < 0.05) are shown in the figure (*, *P* < 0.05; **, *P* < 0.01; *, *P* < 0.001). Different letters indicate significant differences between three crop patterns, and asterisks indicate significant differences between phosphorus levels.**

Intercropping promotes ecosystem multiple services under contrasting P conditions

Intercropping and phosphorus addition significantly stimulated multiple ecosystem services (Fig. 1, and 3). Specifically, the multiple thresholds approach suggested that intercropping delivered more ecosystem services than single crops over a wide range of thresholds (from 25% to 75%) (Fig. 3), while phosphorus only promoted multiple ecosystem services at lower (25%) and medium (50%) levels. PCA showed that the first two principal axes explained 49.5% of the multi-dimensional functional space variation, with DIM 1 and 2 explaining 31.4% and 18.1%, respectively (Fig. S1A). Meanwhile, both intercropping and phosphorus were negatively correlated with DIM1, whereas fertilized intercropping positively correlated with DIM2 (Fig. S1B). Moreover, DIM1 was negatively correlated with diverse key services such as productivity, nutrient cycling, and soil carbon storage (Fig. S1C). Together, our results highlighted the functional trade-offs in long-term disturbed agroecosystems.

Our study goes a step further and provides novel evidence that intercropping can alleviate the dependence of multiple ecosystem services on non-renewable phosphorus fertilization. Our findings showed that unfertilized intercropping better support multiple ecosystem services compared to fertilized single crops, such as soil microbial habitat, plant-soil mutualism, nutrient cycling, and soil carbon storage. More importantly, we found that the effect of intercropping on weighted ecosystem services override the impact by phosphorus addition. Such knowledge is important for us to better predict key ecosystem services in cropland under the shortage of non-renewable fertilizers.

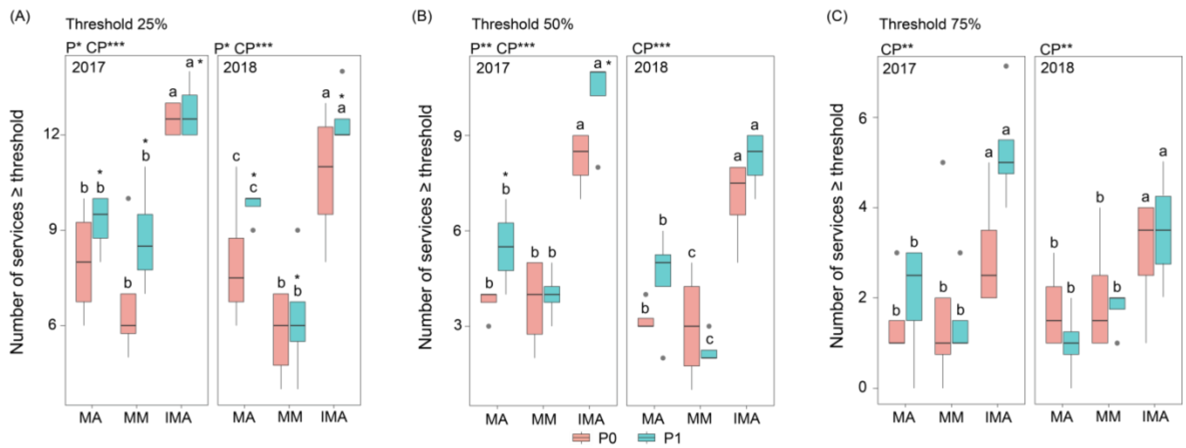

Figure 2 Effects of P, CP, and their interactions on the numbers of services above thresholds of 25% (A), 50% (B), and 75% (C), calculated using the multi-threshold approach. Different letters indicate significant differences between three crop patterns, and asterisks indicate significant differences between phosphorus levels. For abbreviations and analyses of variances see Fig. 1.

Importance of intercropping as a driver of ecosystem services

Intercropping and phosphorus were two important predictors driving multiple ecosystem services in agroecosystems, with intercropping effects overriding those by phosphorus (Fig. 3).

We further found that effects of intercropping on ecosystem services override the impacts by phosphorus in cropland ecosystems (Fig. 4), suggesting that intercropping could contribute to alleviate the dependence of multiple ecosystem services on non-renewable phosphorus fertilization.

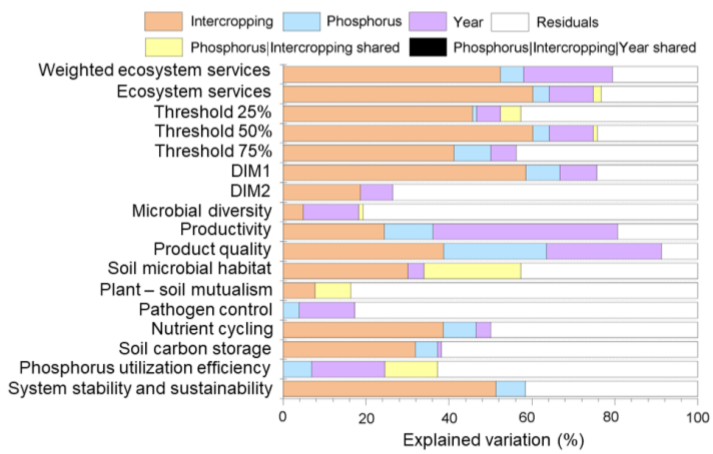

Figure 3 Contribution of the intercropping and phosphorus to ecosystem services. Variation Partitioning Modeling was used to evaluate the unique and shared portions of variation in ecosystem services explained by intercropping, phosphorus, and year. Phosphorus| Intercropping| Year shared = phosphorus| year shared +intercropping| year shared+ phosphorus| intercropping| year shared.

Synergies and trade-offs in the multiple services delivered by agroecosystem under contrasting P conditions

We found some important trade-offs and synergies among multiple ecosystem services in fertilized, unfertilized intercropping and monoculture systems. Specifically, microbial diversity was negatively correlated with phosphorus utilization efficiency services, while positively correlated with plant-soil mutualism. Productivity has been shown to be positively affected by soil microbial habitat (Fig. 4).

Intercropping has been expected to increase crop yields while reducing their environmental footprint (Li et al., 2020; Tilman, 2020; Li et al., 2021). In this study, we found consistently positive correlations between productivity and multiple thresholds services in intercropping, but single crops unremarkable support 75% threshold services at high productivity. This indicates conventional single alfalfa and maize don't support high-level multiple services in agroecosystems and may raise environmental costs (Li et al., 2020). Interestingly, we found tradeoffs between productivity with system stability in single crops, while these services exist synergies in intercropping. We provided evidence that intercropping maintains ecosystem stability based on providing agroecosystems with fundamental productivity services, which are consistent with previous studies (Renard and Tilman, 2019).

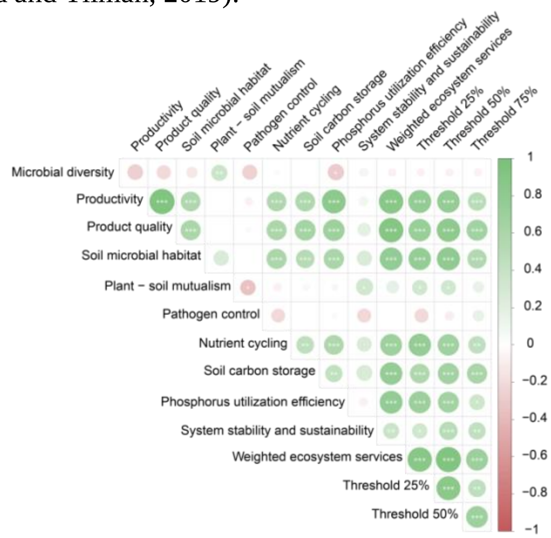

Figure 4 Correlation matrix illustrating the trade-offs and synergies between multiple ecosystem services. The circles' size presents a correlation coefficient. The green and red colors represent positive and negative correlations, respectively. Asterisks were considered to be significant. *, *p* < 0.05; **, *p* < 0.01; *, *p* < 0.001.**

Conclusions

In summary, our work provided novel evidence that intercropping support high levels of agroecosystem multiple services and could contribute to greatly alleviating the dependence of ecosystem services on nonrenewable phosphorus fertilization. Our work further provides new evidence that intercropping optimized the delivery of multiple services and overridden phosphorus, providing the path to agricultural and environmental sustainability. Furthermore, we also identified the important tradeoffs between productivity and biodiversity and further suggested that these tradeoffs can be significantly dampened by intercropping management. This knowledge is critical for supporting multiple agroecosystem services under global mineable phosphorus limitations, which enhances stability in food supply worldwide.

References

Legendre, P., 2007. Studying beta diversity: ecological variation partitioning by multiple regression and canonical analysis. *Journal of Plant Ecology* 1, 3-8.

Li, C., Hoffland, E., Kuyper, T.W., Yu, Y., Zhang, C., Li, H., Zhang, F., van der Werf, W., 2020. Syndromes of production in intercropping impact yield gains. *Nature Plants*., 6, 653-660.

Li, X.F., Wang, Z.G., Bao, X.G., Sun, J.H., Yang, S.C., Wang, P., Wang, C.B., Wu, J.P., Liu, X.R., Tian, X.L., Wang, Y., Li, J.P., Wang, Y., Xia, H.Y., Mei, P.P., Wang, X.F., Zhao, J.H., Yu, R.P., Zhang, W.P., Che, Z.X., Gui, L.G., Callaway, R.M., Tilman, D., Li, L., 2021. Long-term increased grain yield and soil fertility from intercropping. *Nature Sustainability*., 4, 943-950.

Renard, D., Tilman, D., 2019. National food production stabilized by crop diversity. *Nature*., 571, 257-260.

Tilman, D., 2020. Benefits of intensive agricultural intercropping. *Nature Plants*., 6, 604-605.

Wang, L., Delgado-Baquerizo, M., Wang, D., Isbell, F., Liu, J., Feng, C., Liu, J., Zhong, Z., Zhu, H., Yuan, X., Chang, Q., Liu, C., 2019a. Diversifying livestock promotes multidiversity and multifunctionality in managed grasslands. *Proceedings of the National Academy of Sciences* 116, 6187-6192.