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Microbial resistance promotes plant production in a four-decade nutrient fertilization experiment

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ABSTRACT

There is a current lack of mechanistic understanding on the relationships between a soil microbial community, crop production, and nutrient fertilization. Here, we combined ecological network theory with ecological resistance index to evaluate the responses of microbial community to additions of multiple inorganic and organic fertilizers, and their associations with wheat production in a 35-year field experiment. We found that microbial phylotypes were grouped into four major ecological clusters, which contained a certain proportions of fast-growers, copiotrophic groups, and potential plant pathogens. The application of combined inorganic fertilizers and cow manure led to the most resistant (less responsive) microbial community, which was associated with the highest levels of plant production, nutrient availability, and the lowest relative abundance of potential fungal plant pathogens after 35 years of nutrient fertilization. In contrast, microbial community was highly responsive (low resistance) to inorganic fertilization alone or plus wheat straw, which was associated with lower crop production, nutrient availability, and higher abundance of potential fungal plant pathogens. Our work demonstrates that the response of microbial community to long-term nutrient fertilizations largely regulates plant production in agricultural ecosystems, and suggests that manipulating these microbial phylotypes may offer a sustainable solution to the maintenance of field productivity under long-term nutrient fertilization scenarios.

1. Introduction

Nutrient fertilization is typically used to promote crop production. The average percentage of crop yields attributable to fertilizer generally ranges from about 40% to 60% in the USA and England (Stewart et al., 2005). In China, the agricultural consumption of nitrogen fertilizer has reached 28.1 Tg (N) nationally in 2010 (Zhang et al., 2013). However, long-term nutrient fertilization has been shown to result in accumulation of nutrients in soil that subsequently leads to soil acidification, degradation (Coolon et al., 2013), and salinization and NO_3^--N leaching to groundwater (Chen et al., 2004). Moreover, the application of nitrogen fertilizers to soil has resulted in N_2O and NH_3 emission to the atmosphere in the last decades (Liu et al., 2002), with direct emission fluxes of N_2O from farmland of China estimated to be 398 Gg (N) in 1999 (Zhu and Chen, 2002). Consequently, there is an urgent need to optimize fertilization to ensure sustainable food and fiber production for a globally increasing human population.

The use of organic fertilizers, such as livestock manure and crop residue, has been postulated as potential mitigating agents of soil degradation induced by long-term inorganic fertilization (Sanzcobena et al., 2014), however, the ecological mechanism that underpin the mitigation process remain poorly understood. Microbial community is an important driver of rates and stability of food and fiber production across the globe (Delgado-Baquerizo et al., 2017), and could represent

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an organic solution to problems caused by long-term fertilization. Although inorganic fertilization is known to alter microbial community composition (Allison and Martiny, 2008; Ziska et al., 2011; Walsh et al., 2016) and decrease the microbial diversity (Zhou et al., 2016), a recent study suggested that long-term application of combined inorganic fertilizers and livestock manure may contribute to the recovery of soil microbial diversity to pre-inorganic fertilization levels and increase the crop production (Sun et al., 2015), indicating that soil microbes may be key drivers of potential benefits of the combination of inorganic and organic fertilization on crop production.

Microbial community has great influence on plant health and growth. Microbes-plant relationships can be roughly divided into four categories: microbial groups that can increase the supply of mineral nutrients to plant (Nihorimbere et al., 2011); copiotrophic microbes that compete each other for water, nutrients and space, and sometimes threaten crop growth (Berendsen et al., 2012); pathogenic populations associated with the outbreak of plant diseases (Mansfield et al., 2012); and microbial groups that stimulate plant growth indirectly by preventing the growth or activity of pathogens (Nihorimbere et al., 2011). In addition, some microbial taxa could withstand quite challenging conditions (eg., fertilization, drought, etc.), which may play essential roles in microbial ecological fitness (less pathogens) and plant growth (De Vries and Shade, 2013; Geisseler and Scow, 2014). Put it simple, microbial community that maintain stable patterns in response to fertilization could, in theory, help maintain food and fiber production under scenarios of long-term nutrient fertilization. However, more specific ecological hypotheses on how and why the responses of soil microbial community to long-term nutrient fertilization can regulate plant production and long-term experimental evidences are lacking. We hypothesized that the responses of soil microbial community to nutrient fertilization could largely influence the production of food and fiber. For instance, inorganic fertilization may result in the activation of highly responsive (low resistance) microbial community, that could negatively influence plant production through their uptake of rapidly available soil nutrients, and promote plant pathogenesis, due to their fast-growing, opportunistic characteristics (Beattie, 2007; Miller and Fitzsimons, 2011). Conversely, a particular combination of organic and inorganic fertilizers may result in a reduced response (high resistance) of microbial community to nutrient fertilization, which could promote plant production by allowing microbes to uptake slowly-released nutrients (e.g., from manure) and establishing a microbial ecological fitness to minimize plant pathogenesis.

Herein, we used a 35 year-long nutrient fertilization experiment to test the hypothesis: long-term fertilization of organic and inorganic combinations leads to highly resistant microbial community, and promotes plant production through the maintenance of "native" soil microbial community that allow plants to benefit from the slow-release of nutrients and low levels of plant pathogens. Our experiment is based on wheat (*Triticum asdtivum* L.). Globally, wheat is the most commercially important cereal crop accounting for about 25% of cultivation and total food production across the globe (Ray et al., 2013), and contributes to 26% and 21% area of cultivation and total food production in China, respectively (Cai et al., 2002). We used ecological networks (Delgado-Baquerizo et al., 2018) and the ecological resistance index for quantifying the resistance of soil biota to exogenous disturbances (Orwin and Wardle, 2004; De Vries et al., 2012; Shade et al., 2012; Delgado-Baquerizo et al., 2017) to identify ecological clusters of archaea, bacteria, and fungi strongly co-occurring and resistant to different types of nutrient fertilization including NPK inorganic fertilizers, NPK combined with wheat straw, NPK combined with pig manure, and NPK combined with cow manure. We then associated the resistance of these ecological clusters of microbial taxa to the wheat production, nutrient availability and relative abundance of potential fungal pathogens after 35 years of controlled fertilization. The abovementioned nutrient fertilization treatments in this experiment are commonly used across China, and globally, and are critical for understanding the responses of soil microbes and plant production in croplands worldwide. We then identify the role of microbial resistance to nutrient fertilization in regulating plant production by analyzing rhizosphere soil samples which are directly in contact with plants.

2. Materials and methods

2.1. Experimental design

The nutrient fertilization experiment was established in 1982 in Mengcheng County, Anhui Province, China (33°13′N, 116°35′E, 42 m elevation), where there is an annual mean temperature of 14.8 °C and precipitation of 872 mm, and the agricultural soil typical of wheat-soybean crop rotations comprises lime concretion black soil. Five fertilization treatments with four replicates (plots) were arranged in a completely randomized block design (each plot is 70 m^2) (Fig. S1): (1) Control, non-fertilization; (2) NPK, NPK inorganic fertilizers comprising urea (180 kg N ha^{-1} y^{-1}), superphosphate (90 kg P$_2$O$_5$ ha^{-1} y^{-1}) and potassium chloride (86 kg K$_2$O ha^{-1} y^{-1}); (3) NPK + WS, NPK inorganic fertilizers plus wheat straw; (4) NPK + PM, NPK inorganic fertilizers plus pig manure; (5) NPK + CM, NPK inorganic fertilizers plus cow manure. All the wheat straw (about 7500 kg/hm^2) was returned to the filed, pig manure (15,000 kg/hm^2) and cow manure (30,000 kg/hm^2) which had the similar amount of organic carbon with the wheat straw were also added to the field, respectively (Table S1). All the fertilizers were added annually to the field before planting the wheat, and fully mixed with soil. Treatment effects on crop production between 2014 and 2017 were described in Table S2 and Appendix S1.

2.2. Soil sampling

We collected rhizosphere soil samples (by randomly extracting 30–40 wheat individuals and gently shaking off the soil which loosely adhered to the roots, then retaining the soil tightly adhered to roots) during the filling stage of wheat (20th of the April 2017). Soil was passed through 2 mm meshes to remove the impurities (roots, stones etc.), and half of them was stored at −40 °C for subsequent DNA extraction, and the other half was stored at 4 °C for physicochemical analysis.

2.3. Soil physicochemical analysis

Soil pH was determined by pH meter (FE20-FiveEasy pH, Mettler Toledo, German) at a 1:5 soil: distilled water ratio. Soil moisture was measured gravimetrically, where 5 g of fresh soil from dried to a constant weight, and the weight ratio of evaporated water: dried soil was calculated. Soil total carbon (TC) and nitrogen (TN) were determined by combustion (CNS-2000; LECO, St. Joseph, MI, USA), and soil total phosphorus (TP) and total potassium (TK) were extracted using HF–HClO$_4$ digestion, and determined using the molybdenum blue method and flame spectrophotometry methods, respectively. Dissolved organic carbon (DOC) was extracted using distilled water, and determined using a total organic carbon analyzer (Multi N/C 3000, Analytik Jena, Germany). Nitrate (NO$_3^-$-N), ammonium (NH$_4^+$-N) and dissolved total nitrogen (DTN) were extracted by 2 mol·L^{-1} KCl, and determined by a continuous flow analytical system (San++System, Skalar, Holland). Dissolved organic nitrogen (DON) was calculated using the following formula: DON = DTN − NH$_4^+$-N − NO$_3^-$-N. Soil available phosphorus (AP) was extracted by using 0.5 mol·L^{-1} NaHCO$_3$, and determined using the molybdenum blue method. Soil available potassium (AK) was extracted using 1 mol·L^{-1} CH$_3$COONH$_4$, and determined using flame photometry (FP640, INASA, China). Treatment effects on physicochemical properties were described in Table S3 and Appendix S1.

2.4. High-throughput Sequencing and bioinformatics analysis

Soil microbial DNA was extracted from 0.5 g of fresh soil using a Fast

DNA SPIN Kit (MP Biomedicals, Santa Ana, CA). Archaeal and bacterial 16S rRNA genes were amplified using primer pairs 524F-10-ext (5′-TGYCAGCCGCCGCGGTAA-3′) with Arch958-modR (5′-YCCGGCGTT-GAVTCCAATT-3′) (Baker et al., 2003), and 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) with 907R (5′-CCGTCAATTCCTTTGAGTTT -3′) (Biddle et al., 2008), respectively; and the ITS1 fungal region was amplified using primer pairs ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA -3′) with 2043R (5′-GCTGCGTTCTTCATCGATGC -3′) (Bokulich and Mills, 2013). The research sequences were submitted to the NCBI Sequence Read Archive (SRA) with accession number SRP126794 (https://www.ncbi.nlm.nih.gov/sra/SRP126794).

We obtained the high quality sequenced data of archaea (N = 384,250), bacteria (N = 508,260), and fungi (N = 480,360) after the removal of short fragments (<200bp) and low quality sequences (average quality scores <25 reads) by using Quantitative Insight into Microbial Ecology (QIIME-1.9.1) pipeline (http://qiime.sourceforge.net/) (Caporaso et al., 2010). OTUs were generated based on a 97% level of similarity using UCLUST (Edgar, 2010). We used the Greengenes database (http://greengenes.lbl.gov/) for the taxonomic identity of each phylotype of archaea and bacteria; and we used the UNITE database for the taxonomic identity of fungi. Treatment effects on microbial taxa (Table S4) and alpha diversity (Table S5) were described in Appendix S2.

We further analyzed the functional structures of fungal community in each treatment, the functional traits of fungal species were obtained from the online application FUNGuild (http://www.stbates.org/guilds/app.php) and the published researches (Dean et al., 2012; Nguyen et al., 2016). Of note, we kept 875 OTUs with confidence ranking of "Highly Probable" and "Probable" to reach the high accuracy, which account for 41.3% of total fungi OTUs. We only focused on the functional guilds of potential animal pathogen (115 OTUs), potential plant pathogen (234 OTUs), dung saprotroph (87 OTUs) and endophyte (59 OTUs), which were relevant to plant health and potential risks of the agricultural ecosystems. Treatment effects on relative abundance of key microbial functional groups were presented in Table S12, Fig. S3 and Appendix S3.

2.5. Co-occurrence network analysis

We constructed the co-occurrence network and identified four ecological clusters of strongly associated OTUs by using default parameters from the interactive platform "Gephi" (Delgado-Baquerizo et al., 2018). We focused on those microbial phylotypes (i.e., operational taxonomic units or OTUs) accounting for more than 80% of the relative abundance of archaea, bacteria and fungi, respectively. The archaeal, bacterial and fungal OTUs were merged into an abundance table, which including 248 archaeal, 3097 bacterial and 357 fungal OTUs. Then we calculated all pair-wise spearman correlations between OTUs. We only focused on the positive correlations which are critical for co-occurrence patterns and will lead to ecological clusters of microbial phylotypes sharing similar environmental preferences (Barberán et al., 2014; Delgado-Baquerizo et al., 2018). We cut off the correlations with Spearman's coefficient was of less than 0.65, and P value of more than 0.01, and focused on the OTUs strongly co-occurring with each other within the microbial community. The main ecological clusters in the network were visualized with the Gephi (https://gephi.org/) (Bastian et al., 2009), and the relative abundance of each ecological cluster was calculated by averaging the standardized relative abundances (z-score) of the species that belong to it (Delgado-Baquerizo et al., 2018).

2.6. Microbial community resistance indices

We calculated the Orwin Wardle resistance indices (Orwin and Wardle, 2004) for the relative abundance of ecological clusters as:

$$Resistance = 1 - \frac{2|D_0|}{(C_0 + |D_0|)}$$

where C_0 is the value of the Control and D_0 is the difference between the nutrient fertilization treatments and the Control (Fig. S1). The advantages of the Orwin Wardle resistance index include: (1) it is standardized by the control; (2) it ranges between −1 (low resistance) and +1 (maximal resistance), even when extreme values are encountered (Orwin and Wardle, 2004).

2.7. Statistical analysis

We used one-way ANOVA and post-hoc analyses to test for treatment differences in soil variables, crop production, microbial diversity, the relative abundance of main microbial taxa, relative abundance of ecological clusters from networks, and the resistance indices of ecological clusters. The relationships between the resistance indices of ecological clusters (Module #1–4) and the wheat production, the potential plant potentials, and the available nutrients (nitrogen, phosphorus, and potassium) were tested by linear regressions using SPSS 21. The variations of archaea, bacteria and fungi community between treatments (based on the Bray-Curtis distance matrix) were tested using the ADONIS and non-metric multidimensional scaling (NMDS) in 'vegan' package in R software.

2.8. Structural equation modeling

We used structural equation model (SEM) (Grace, 2006) to evaluate the direct and indirect effects of nutrient fertilizations and resistance indices of main ecological clusters on available nutrients, potential plant pathogens, and wheat production. SEM allows the partitioning of these associations, and is critical to test for the association between microbial resistance and plant production while considering multiple other factors simultaneously. In all cases, the treatments (NPK; NPK + WS; NPK + PM; NPK + CM) were categorical variables with two levels: 1 (a particular treatment) and 0 (remaining considered treatments + others). As a few of variables were not normally distributed, we combined bootstrapping to test the probability that a path coefficient differs from zero. To aid interpretation of the SEM, the standardized total effects (STEs) were calculated for the treatments, available nutrients (available nitrogen, available phosphorus and available potassium), and resistance indices on wheat production. All the SEM analyses were conducted by using IBM SPSS Amos 21 (Chicago, IL: Amos Development Corporation).

3. Results

3.1. Microbial composition of each ecological cluster

Soil microbial phylotypes were grouped into four major ecological clusters (Module #1–4), which were formed by strongly co-occurring archaea, bacteria, and fungi taxa (Fig. 1A; Table S7). Module #1 included phylotypes from archaeal family *SAGMA-X* (12.5%) and *Nitrososphaeraceae* (29.1%) belonging to *Cenarchaeales* and *Nitrososphaerales*, respectively. Module #1 also contained phylotypes from bacterial phyla *Acidobacteria* (8.41%) (e.g., *Acidobacteriaceae*, *Koribacteraceae*, and *Solibacteraceae*), *Bacteroidetes* (3.42%) (e.g., *Sphingobacteriaceae*), *Betaproteobacteria* (e.g., *Burkholderiaceae* and *Oxalobacteraceae*), and *Gammaproteobacteria* (3.55%) (e.g., *Xanthomonadaceae*). For the fungal family, *Xenopolyscytalum* (2.95%), *Nectriaceae* (3.40%), *Chaetomiaceae* (4.82%), and *Herpotrichiellaceae* (2.52%) were highly enriched in the Module #1. Module #2 included phylotypes from bacterial family *Flavobacteriaceae* (1.26%) and fungal family *Pyronemataceae* (10.3%). Module #3 was the smallest ecological cluster and dominated by the fungal family *Xylariales* (2.16%). Module #4 consisted of archaeal family *Nitrososphaeraceae* (33.8%), bacterial families
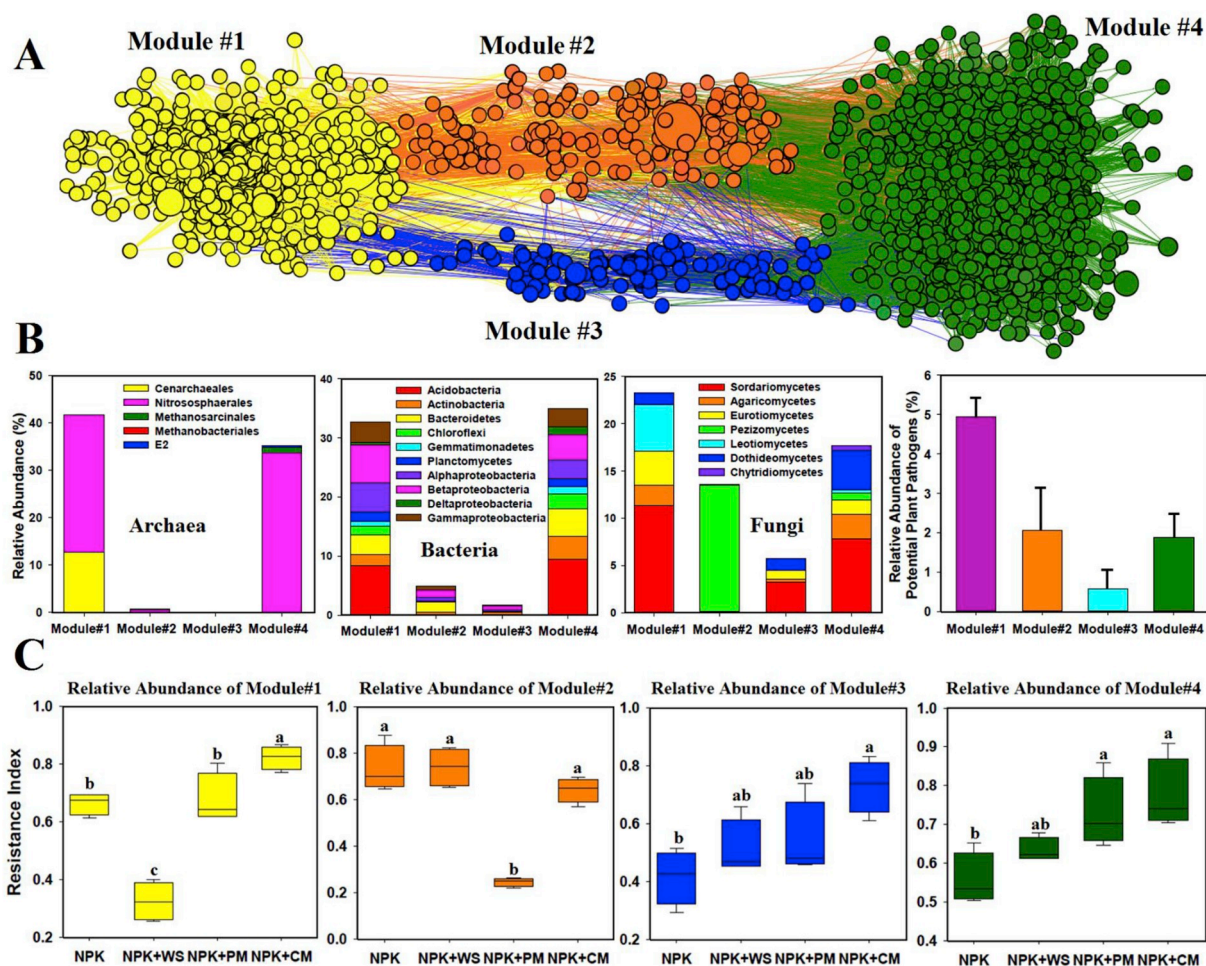
Fig. 1. Microbial ecological clusters and resistance indices. (*A*) Network diagram of ecological clusters (Modules) with nodes of archaeal, bacterial, and fungal OTUs. (*B*) The relative abundance of dominant taxa and potential plant pathogens in each ecological cluster. (*C*) Fertilization effects on resistance indices of the relative abundance taxa in the four principal ecological clusters (Modules). The resistance index ranges from −1 (minimum resistance) to +1 (maximum resistance). NPK: applications of nitrogen (N), phosphorous (P), and potassium (K); NPK + WS: NPK with wheat straw; NPK + PM: NPK with pig manure; and, NPK + CM: NPK with cow manure.

Chitinophagaceae (2.03%) and *Comamonadaceae* (2.03%), and fungal family *Davidiellaceae* (3.60%). The phylotypes included in each module were available in Table S8, Table S9 and Fig. 1 *B*.

3.2. Ecological clusters resistant to nutrient fertilization

The relative abundance of Module #1, #3, and #4 in the long-term NPK + cow manure fertilization (Module #1: 0.14; Module #3: 0.37; Module #4: 0.16) has no significant difference with Control (Module #1: 0.11; Module #3: 0.39; Module #4: 0.16); while the relative abundance of Module #1–4 was significantly decreased in the long-term NPK + cow manure fertilization (Module #1: 0.11; Module #2: 0.40; Module #3: 0.39; Module #4: 0.16) when compared with long-term NPK inorganic fertilization (Module #1: 0.18; Module #2: 0.47; Module #3: 0.41; Module #4: 0.20). The relative abundance of Module #3 was significantly increased in the long-term NPK inorganic fertilization (0.41), NPK + wheat straw (0.45), and NPK + pig manure fertilization (0.48) when compared with Control (0.37) (Table S10). The application of NPK + cow manure led to the most resistant (less responsive) microbial community (Module #1: 0.82; Module #2: 0.69; Module #3: 0.73; Module #4: 0.75) when compared with other fertilization treatments (Fig. 1*C*; Table S11). On the contrary, the application of NPK + pig manure significantly decreased the resistance of taxa (relative abundance) within Module #2 (0.25); the application of NPK + wheat straw

lead to reductions in the resistance of Module #1 (0.38); and NPK inorganic fertilization significantly decreased the resistance of Module #3 (0.47) and Module #4 (0.58) when compared with other treatments (Fig. 1*C*; Table S11).

3.3. Linking ecological cluster resistance to nutrient fertilization with plant production

In general, the resistance of the relative abundance of ecological clusters (Module #1, Module #3, and Module #4) to nutrient fertilization was positively correlated to plant production, and to the availability of N, P or K. However, the resistance of the relative abundance of these clusters was negatively associated with the abundance of potential plant pathogens (Fig. 2; Table S14). Thus, our results indicate that increases in the resistance of these ecological clusters (low change in relative abundance in response to nutrient fertilization) matched with the lowest relative abundance for potential plant pathogens (Fig. 2), and positively correlated with important soil nutrients including available N (nitrate) (Module #1), P (Module #1, #3), and K (Module #1, #4) (Fig. 2; Table S14). Put simply, those treatments where nutrient fertilization resulted in high wheat production, greater nutrient availability and lower pathogen abundance matched with low responsive (high resistance) ecological clusters to nutrient fertilization –the relative abundance of taxa within these clusters did not change significantly in
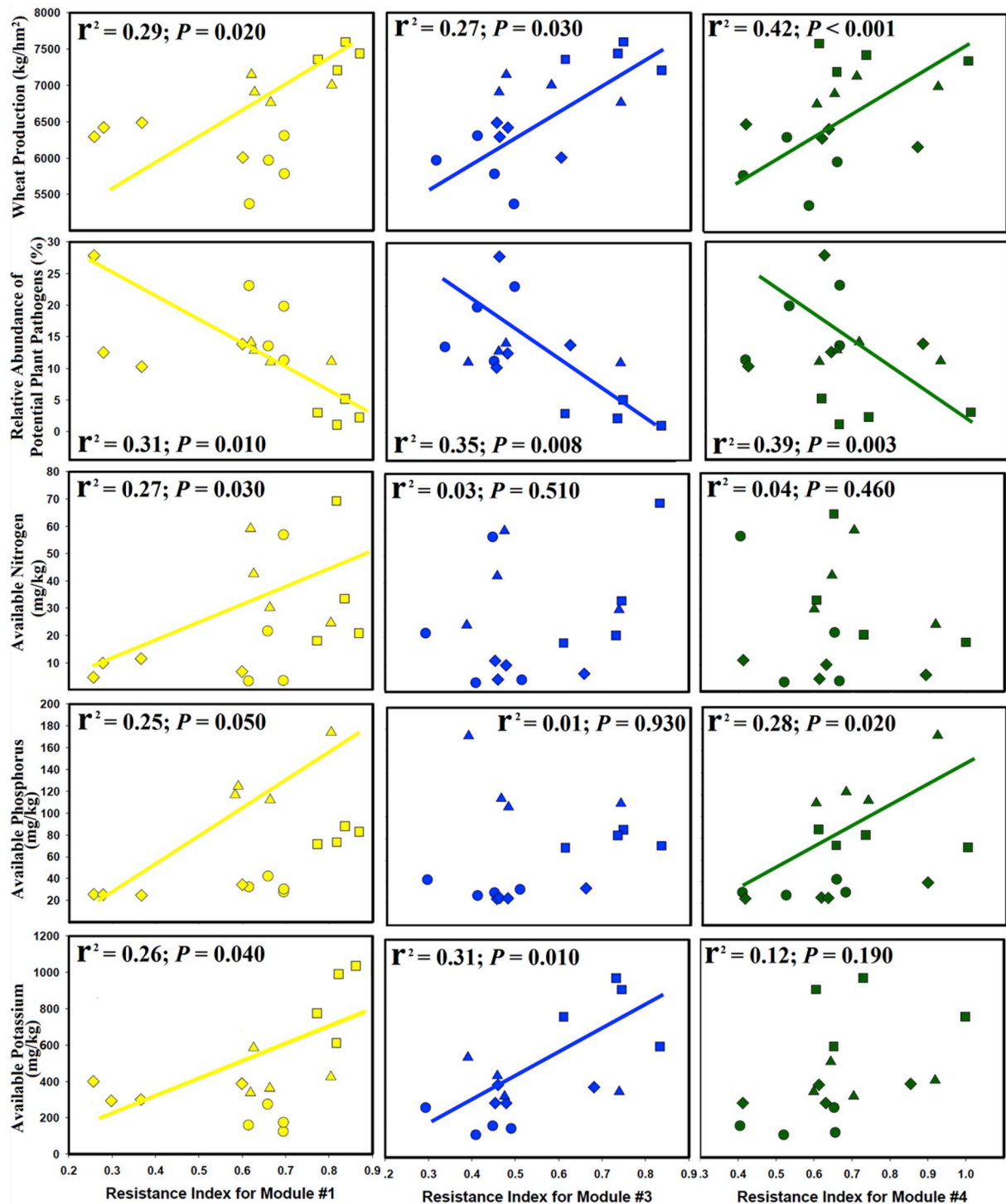
Fig. 2. Regressions between the resistance of ecological clusters to nutrient fertilization and wheat production, relative abundance of plant pathogens, available nutrients in a 35-year experiment. NPK: applications of nitrogen (N), phosphorous (P), and potassium (K) (●); NPK + WS: NPK with wheat straw (◆); NPK + PM: NPK with pig manure (▲); and, NPK + CM: NPK with cow manure (■).

response to the nutrient fertilization treatments. Importantly, increases in the relative abundance of these ecological clusters were also negatively correlated with wheat production (Table S13). Thus, nutrient fertilization treatments that resulted in increases in the relative abundance of these ecological clusters (especially Module #1, Module #3, and Module #4) (therefore, in low resistance to nutrient fertilization), were associated with lower wheat production. With one exception, the resistance of the relative abundance of Module #2 had no significant correlations with plant production, potential plant pathogens and the

availability of N or K (Fig. S4).

3.4. Indirect effects of nutrient fertilization on microbial resistance and plant production

After ecological clusters that were resistant to different types of nutrient fertilization had been identified, we then conducted structural equation models (SEM) to evaluate whether there were indirect treatment effects on wheat production due to nutrient fertilization mediated

changes in microbial resistance. The SEM explained more than 88% of the variation in wheat production (Fig. 3A), among which available nutrients (nitrate nitrogen, available phosphorus, and available potassium) were directly positively correlated with crop production, while relative abundance of potential plant pathogens was negatively correlated with wheat production (Fig. 3). Importantly, the resistance of the relative abundance of most ecological clusters (Module #1, #3, #4) was positively correlated with plant production (Fig. 3B) after controlling for multiple environmental factors. To support our hypothesis, the

resistance of ecological clusters to particular types of nutrient fertilization (e.g., NPK + cow manure) indirectly, but positively regulated plant production through the increased availability of nutrients and the reduction in relative abundance of potential fungal plant pathogens.

4. Discussion

In our study, each ecological cluster contained a certain proportions of fast-growers, copiotrophic groups, and potential plant pathogens. For
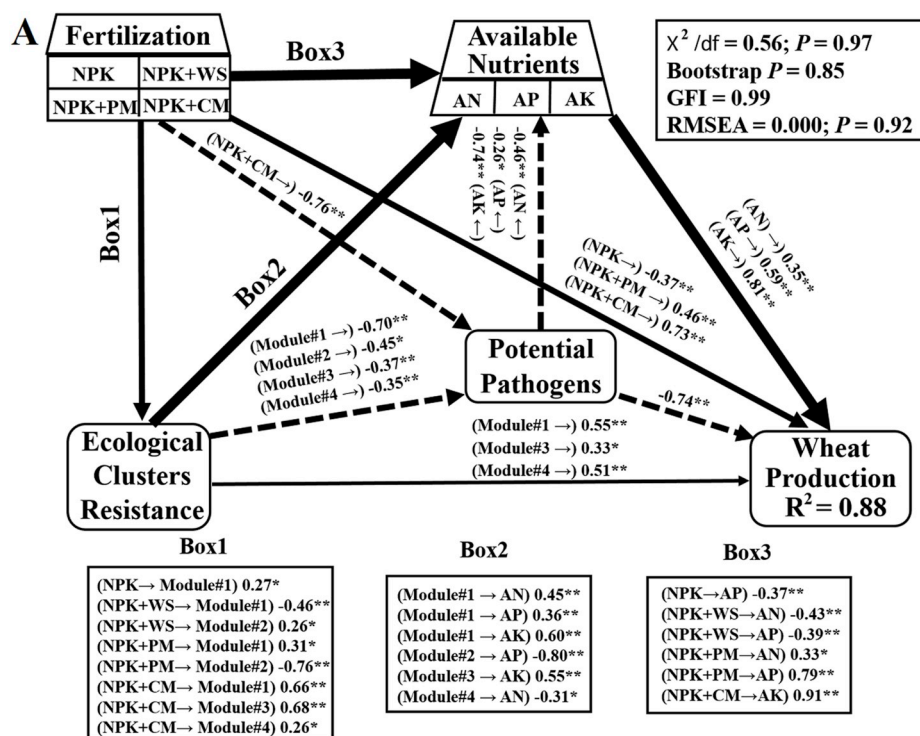

Fig. 3. (A) Structural equation model describing the direct and indirect effects of nutrient fertilization treatment and resistance indices of the ecological clusters (Module #1 to #4) on available nutrients, potential plant pathogens, and wheat production. Arrow width is proportional to the strength of the relationship. Solid line: positive correlation; dashed line: negative correlation. Numbers above/below the arrow lines are indicative of the correlations. The proportion of variance explained (R^2) appears alongside wheat production in the model. Goodness-of-fit statistics for each model are shown in the up right corner (χ^2, Chi-square; df, degrees of freedom; P, probability level; RMSEA, root mean squared error of approximation). Significance levels of each predictor are *$P < 0.05$, **$P < 0.01$. (B) Total standardized effects (sum of direct and indirect effects) in SEM on wheat production from nutrient fertilization, available nutrients, potential pathogens, and resistance of main ecological clusters. NPK: applications of nitrogen (N), phosphorous (P), and potassium (K); NPK + WS: NPK with wheat straw; NPK + PM: NPK with pig manure; and NPK + CM: NPK with cow manure AN: nitrate nitrogen (NO_3^--N); AP: available phosphorus; and, AK: available potassium.
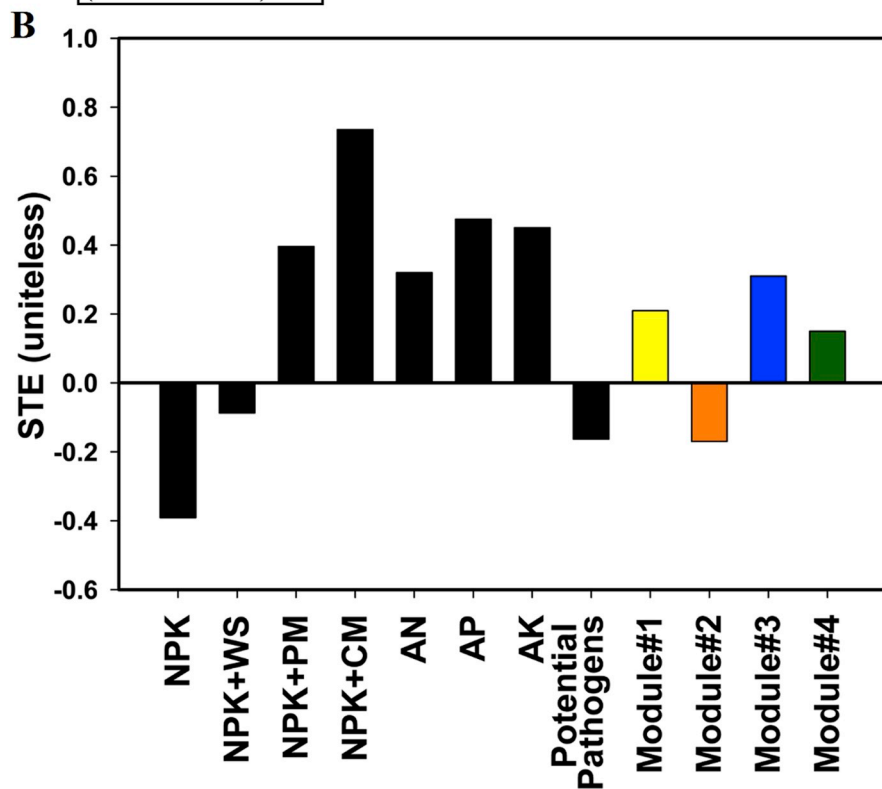
example, Module #1 was dominated by multiple phylotypes of adversity adaptors such as *Cenarchaeale* that is a kind of extremophile and could adapt to the strong reduction or high salinity conditions (Reysenbach et al., 2001; Shi et al., 2010), and *Acidobacteria* that can adapt to the acidification conditions (Lennon and Jones, 2011). The fast-growers (eg., *Oxalobacteraceae* and *Xanthomonadaceae*) (Green et al., 2007) and potential pathogenic fungi *Xenopolyscytalum* (Tyub et al., 2018), *Nectriaceae* (Lombard et al., 2015), *Chaetomiaceae* (Violi et al., 2007), and *Herpotrichiellaceae* (Crous et al., 2007) were also highly enriched in the Module #1. Meanwhile, Module #2 and Module #3 were dominated by opportunistic pathogens such as *Flavobacteriaceae* (Bernardet et al., 2002), *Pyronemataceae* (Perry et al., 2007) and *Xylariales* (Yee et al., 2009). Module #4 consisted of plant-growth-promoting and fast-growing phylotypes from *Chitinophagaceae* (Madhaiyan et al., 2015) and potential pathogens from *Comamonadaceae* (Willems, 2014) and *Davidiellaceae* (Martin et al., 2012).

Different fertilization treatments resulted in more or less responsive microbial community (in terms of their relative abundance). The application of NPK + cow manure led to the most resistant (less responsive) microbial community, which was associated with the highest levels of plant production, nutrient availability, and lowest relative abundance of potential fungal plant pathogens following 35 years of nutrient fertilization. These findings suggest that the resistance of microbial community to nutrient fertilization is a critical factor in regulating plant production in an economically important crop system. Several mechanisms may explain the role of microbial resistance to nutrient fertilization in the promotion of plant production. First, low responsive microbial community may lead to higher availability of nutrients for plants, as supported by our findings of direct and positive correlations between ecological cluster resistance and available nutrients (nitrate nitrogen, available phosphorus, and available potassium), that indicate resistant microbial community facilitate plants getting more nutrients and less competition from microbial species. On the contrary, we also found that inorganic (NPK) or other combination of inorganic and organic fertilizations (NPK + wheat straw) can promote highly responsive microbes (e.g., fast-growers or copiotrophic microbes) that would consume greater amounts of resource and outcompete plants for colonization sites and nutrients (Weyens et al., 2009; Berendsen et al., 2012). For example, the relative abundance of Module #1 and Module #4 that were enriched by many bacterial fast-growers such as *Oxalobacteraceae*, *Xanthomonadaceae* (Green et al., 2007) and *Chitinophagaceae* (Madhaiyan et al., 2015) was higher in the treatments of NPK and NPK + wheat straw, indicating that these microbial taxa might benefit from long-term applications of NPK inorganic fertilizers or NPK + wheat straw. More importantly, our results suggest that microbial community within these ecological clusters which are highly responsive to nutrient fertilization would outcompete plants for nutrients, and threaten the plant growth.

Second, low responsive microbial community may lead to lower relative abundance of potential fungal plant pathogens. Long-term application of different fertilizers results in significant variations in microbial community structure and function (Marschner et al., 2003; Van Der Heijden et al., 2008), and resistant microbial community may suppress internal fluctuations to maintain microbial ecological fitness and control the relative abundance of potential plant pathogens (Kobayashi and Crouch, 2009). In our study, the main ecological clusters contained multiple potential bacterial and fungal plant pathogens (e.g., bacterial family: *Burkholderiaceae*, *Flavobacteriaceae*, and *Comamonadaceae*; fungal family: *Xenopolyscytalum*, *Xylariales*, *Pyronemataceae*, *Nectriaceae*, and *Davidiellaceae*) (Bernardet et al., 2002; Martin et al., 2012; Coenye, 2014; Willems, 2014; Lombard et al., 2015; Tyub et al., 2018). We found that the long-term applications of NPK inorganic fertilization and NPK + wheat straw resulted in increases in the relative abundance of potential plant pathogens, which might trigger plant disease, and could lead to negative effects on the crop production (Abawi and Widmer, 2000). We would like to stress the reader that, at least partially,

the higher relative abundance of potential plant pathogens in NPK + wheat straw may associated with the combined effect of potential pathogen inoculation (from wheat straw) and the biotic interactions between resistant microbes and pathogens. Unlike NPK + wheat straw, our results indicate that the long-term application of NPK + cow manure decreases the risks from potential pathogens, which may have a long-term positive effect on wheat production. Although the interactions between legacy effects from fertilizers on fungal pathogens and other soil microbial communities effects are difficult to control, further investigations need to be done in the future to address this important point.

Taken together, our results provide mechanistic understanding on how microbial community regulates the effects of long-term nutrient fertilization on plant production for one of the most commercially important cereal crops. Such information is vital to maintain food production in response to a continually growing human population which will reach more than 9 billion by the middle of this century (Godfray et al., 2010). Our study demonstrates that the resistance of microbial community to nutrient fertilization treatment regulates long-term plant production through the control of availability of nutrients and the relative abundance of potential plant pathogens. Long-term addition of NPK + cow manure fertilizers was the most effective management for increasing microbial resistance and plant production. Low responsive (more resistant) microbial community (in terms of relative abundance) to nutrient fertilization was associated with higher nutrient availability, lower relative abundance of potential plant pathogens, and higher plant production. These findings highlight the importance of microbial resistance in regulating the production of economically and ecologically important crops. The lesson to learn here, is that microbial resistance indirectly drive the effects of nutrient fertilization on plant production, and that this is totally dependent on the types of fertilizers applied, therefore, management practices targeting resistant microbial community are realistic and advisable.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.107679.

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