Rhizosphere 9 (2019) 38–46

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Maize plant growth response to whole rhizosphere microbial communities in different mineral N and P fertilization scenarios

Dante A. López-Carmona[a], Alejandro Alarcón[b], Esperanza Martínez-Romero[c], Juan José Peña-Cabriales[d], John Larsen[a,*]

[a] Instituto de investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro 8701, Col. Ex Hacienda de Sán José de la Huerta, 58190 Morelia, Michoacan, Mexico
[b] Colegio de Postgraduados Campus Montecillo, Carretera México-Texcoco Km. 36.5, 56230 Texcoco, Estado de Mexico, Mexico
[c] Instituto de Biotecnología, Universidad Nacional Autónoma de México, Avenida Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos, Mexico
[d] Departamento de Biotecnología y Bioquímica, CINVESTAV, Km. 9.6 Libramiento Norte Carretera Irapuato-León, 36821 Irapuato, Guanajuato, Mexico

ARTICLE INFO

Keywords:
Agroecology
Maize
Mineral fertilization
Rhizosphere
Plant growth promoting microorganisms

ABSTRACT

With the objective to examine maize plant response to whole rhizosphere microbial communities in terms of growth and nutrition, we performed a greenhouse pot experiment with full mineral fertilization except for N and P, which were applied in a fully factorial set-up (Without, with N, with P and with N + P). Rhizosphere microbial communities were measured in terms of mycorrhiza formation and biomarker fatty acids. Whole rhizosphere microbial communities promoted plant growth with single N fertilization (P limitation), whereas negative plant growth responses were observed without fertilization (N and P limitation), single P fertilization (N limitation) and dual N and P fertilization (no nutrient limitation). Whole rhizosphere microbial communities increased and decreased shoot N content without and with N fertilization, respectively. On the other hand whole rhizosphere microbial communities increased shoot P content both with and without P fertilization, though more without P. The observed plant growth and shoot nutrient response to whole rhizosphere microbial communities seems to be linked to the root association with arbuscular mycorrhizal fungi (AMF) and abundance of rhizosphere microorganisms. In conclusion, our results show that maize plant response to whole rhizosphere microbial communities in terms of growth and nutrition is strongly associated with N and P fertilization balance.

1. Introduction

Plant roots naturally associate with plant growth promoting microorganisms, such as AMF and rhizobacteria (Philippot et al., 2013). Conservation and management of these rhizosphere microorganisms has been suggested key to increase crop N and P use efficiency (Drinkwater and Snapp, 2007; Richardson et al., 2009).

Among plant growth promoting microorganisms AMF are important for crop nutrition connecting the root with the soil phase via the formation of an extensive mycelial network providing valuable mineral nutrients especially the less mobile ones like P (Smith and Smith, 2011). Regarding N nutrition rhizobacteria are of special importance since they can acquire N from the atmosphere (Gutiérrez-Zamora and Martínez-Romero, 2001).

Most studies on plant growth promoting microorganisms focus on single species (Dobbelaere et al., 2002; Harman et al., 2004; Leggett et al., 2015; Sarabia et al., 2018) or consortia with microorganisms from different functional groups (Vázquez et al., 2000; Berta et al., 2013; Larsen et al., 2017), whereas information on plant growth response to whole rhizosphere microbial communities is limited, which is a major constraint for successful integration of these plant beneficial microorganisms in agroecosystems.

Maize is worldwide a basic crop for human consumption and animal fodder, which depends on high input of mineral fertilizers (Ranum et al., 2014). The maize root microbiome has received increasing interest to unravel its potential to improve maize growth and nutrition (Peiffer et al., 2013; Walters et al., 2018), but information on how agricultural practice affect the contribution of rhizosphere microorganisms on maize growth and nutrition is limited.

Agricultural practices such as crop rotation, tillage, pest management and fertilization are all essential components for crop production, which should be considered when conserving and managing native populations of plant growth promoting microorganisms in agroecosystems (Larsen et al., 2014). Especially application of mineral P fertilizers

* Corresponding author:.
E-mail address: jlarsen@cieco.unam.mx (J. Larsen).

https://doi.org/10.1016/j.rhisph.2018.11.004
Received 12 November 2018; Accepted 12 November 2018
Available online 17 November 2018
2452-2198/ © 2018 Elsevier B.V. All rights reserved.

Table 1
P values from analysis of variance of the main factors inoculum, N and P fertilization and their interactions for the variables shoot and root dry weight, shoot nutrients, response to rhizosphere microorganisms and AMF root colonization (n = 4).

	Dry weight		Shoot nutrients				Response to rhizosphere microorganisms			Root colonization
	Shoot	Root	N	P	Total P	Total N	Plant growth	Shoot P	Shoot N	AMF
Inoculum (I)	***	**	***	0.517	***	***	**	**	0.1809	0.4048
P	***	***	***	***	***	***	***	***	***	**
N	***	***	***	***	***	***	***	***	0.5261	***
I × P	***	***	***	***	***	***	0.0784	***	***	***
I × N	*	0.8116	**	0.8971	*	**	0.3646	0.6832	0.2967	**
P × N	***	***	***	***	***	***	***	***	0.586	0.1022
I × P × N	***	*	*	*	**	**	0.1861	0.3084	0.1556	0.3364

* $p \leq 0.05$;
** $p < 0.01$;
*** $p < 0.001$.

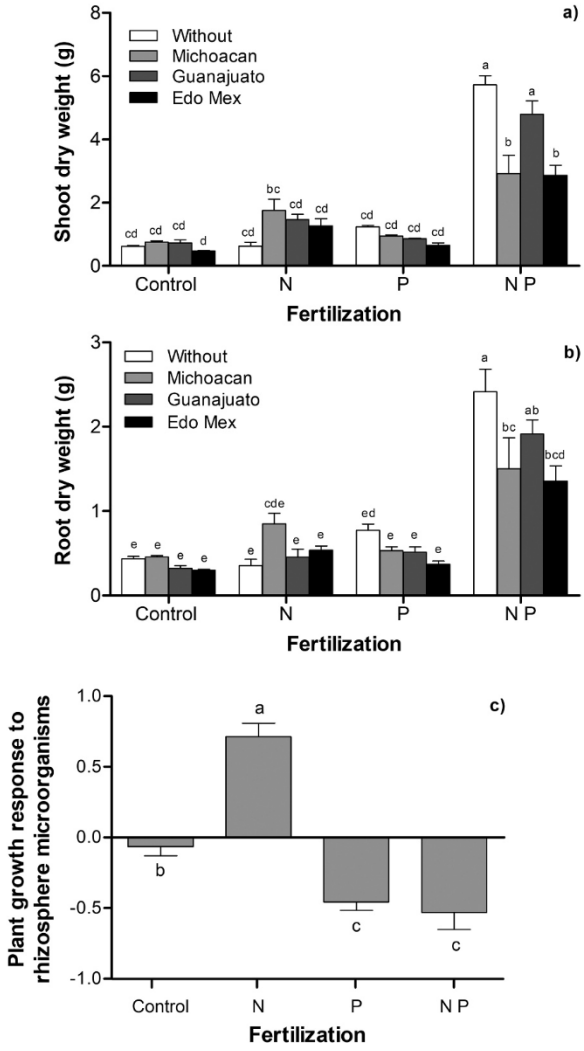

Fig. 1. Shoot (a) and root (b) dry weight and plant growth response to rhizosphere microorganisms (c) of eight-week-old maize plants without microbial inoculation or inoculation with whole rhizosphere microbial communities obtained from Michoacán, Guanajuato and Estado de México in combination with different mineral fertilization scenarios all with basic fertilization differing only in application of N and P: Without, with P, with N and with N and P). Values represent treatment means with standard error bars (n = 4). Treatments with different letters are significantly different according to post hoc ANOVA Tukey test ($p \leq 0.05$).

can have negative effects on the functioning of mycorrhizal associations (Bhadalung et al., 2005; Treseder and Allen, 2002) and may result in plant growth suppression, when the cost of maintaining the mycorrhizal symbiosis in terms of host photosynthates exceeds the benefits from improved AMF hyphal P uptake (Johnson and Graham, 2013).

Here we examined the contribution of three field populations of whole rhizosphere microbial communities on plant growth and N and P nutrition of maize plants subjected to four contrasting mineral fertilization settings all with full mineral fertilization other than N and P: 1) Control without N and P, 2) with N, 3) with P and 4) with N and P. We tested the hypothesis that plant growth and nutrient promotion by whole rhizosphere microbial communities will occur mainly under N or P limiting conditions.

2. Materials and methods

2.1. Experimental design

The experiment had a randomized three-factorial design: 1) Whole communities of rhizosphere microorganisms (Without and with inoculum from Michoacán, Guanajuato and Estado de México), 2) Nitrogen fertilization (Without and with) and 3) Phosphorus fertilization (Without and with). Each of the 16 treatments had 4 replicates resulting in a total of 64 experimental units.

2.2. Biological materials

Sampling of maize field rhizosphere soil was performed in November 2013 in the senescence plant growth phase in fields with rainfed maize in Michoacan, Guanajuato and Estado de Mexico of conventional small holders. Geographic references and soil characteristics of the three fields are presented in Supplementary Table 1. In each field, an X sampling design was employed with the four extreme points and the center as the sampling points resulting in five samples. From each of the five sampling points complete 30 × 30 × 30 cm blocks of soil were excavated with the plant stem as the centre and transferred to laboratory facilities for subsequent sample processing and analyses. Stones, large debris and macro fauna were removed from the soil, which thereafter was homogeneously mixed. A composite sample was made by mixing rhizosphere soil from all five blocks from each site.

Soil used for propagation of inoculum of rhizosphere microorganisms and in the main experiment was collected from the experimental field station of the Chapingo Autonomous University, Campus Morelia, Michoacán, Mexico (19 °41′9.43″N, 101 °14′18.34″W). Soil texture was clayish (53.2% clay, 27.3% silt and 19.5% sand) and contained 2.7% organic matter, 23.2 mg kg^{-1} inorganic nitrogen, 5.8 mg kg^{-1} available

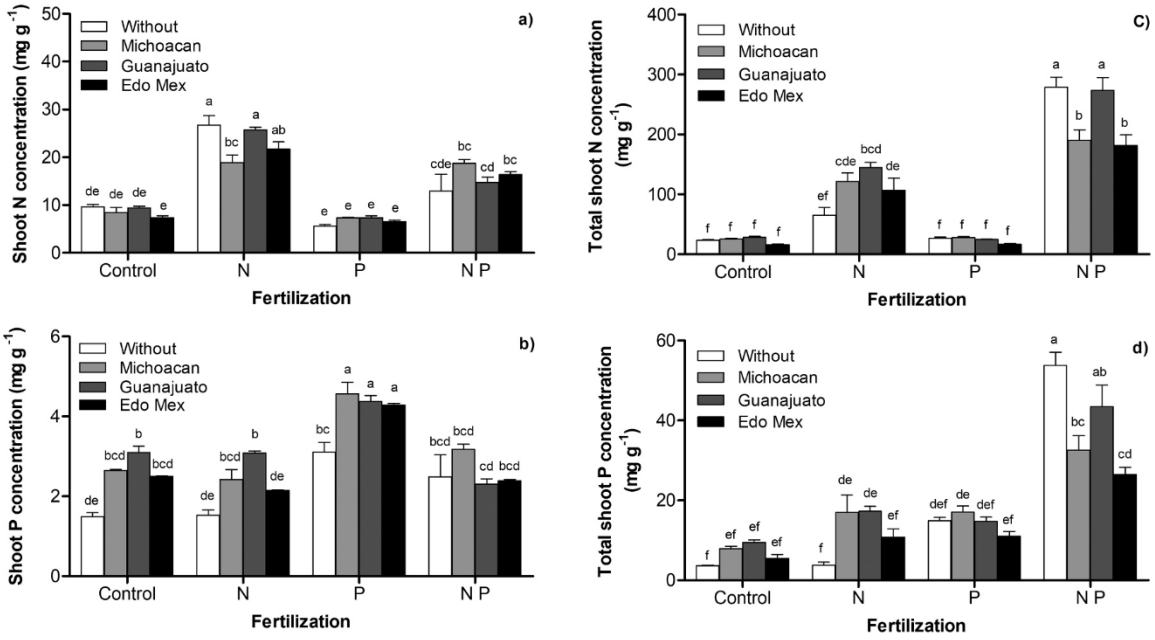

Fig. 2. Shoot N (a) and P (b) concentration and total shoot N (c) and P (d) content of eight-week-old maize plants without microbial inoculation or inoculation with whole rhizosphere microbial communities obtained from Michoacán, Guanajuato and Estado de México in combination with different mineral fertilization scenarios all with basic fertilization differing only in application of N and P: Without, with P, with N and with N and P). Values represent treatment means with standard error bars ($n = 4$). Treatments with different letters are significantly different according to post hoc ANOVA Tukey test ($p \leq 0.05$).

phosphorus (Olsen P) and pH (H_2O) was 7.3. Soil was mixed with quartz sand (1:1, w:w) and disinfected in an autoclave (1 h, 120 °C).

Inoculum with communities of whole rhizosphere microorganisms was propagated using maize (*Zea mays* L, DK2041) as trap plants grown under greenhouse conditions. Maize seeds were sown in 3 L pots with 3 kg sterile soil:sand substrate (1:1, w/w) inoculated with maize field rhizosphere soil (10%) and without inoculation for the control treatment. Prior sowing all basic fertilizers except P were applied in the following amount (mg kg^{-1}): K_2SO_4 (105), $CaCl_2$ (105), $CuSO_4{\cdot}5H_2O$ (2.94), $ZnSO_4{\cdot}7H_2O$ (7.56), $MnSO_4{\cdot}H_2O$ (14.7), $CoSO_4{\cdot}7H_2O$ (0.55), $MgSO_4{\cdot}7H_2O$ (63), and $Na_2MoO_2{\cdot}7H_2O$ (0.25). P fertilization was omitted in order to promote formation of the maize mycorrhiza association. N was applied as NH_4NO_3 corresponding to 50 mg N every second week starting two weeks after sowing and ending one week before harvesting resulting in a total of 200 mg N applied. Greenhouse temperature was approximately 15 °C at night and 25–30 °C during daytime. Plants were watered by weight on a daily basis to maintain 70% of the water holding capacity. Fifteen weeks after sowing when maize plants had reached the flowering stage plants and soil were left to dry for two weeks in the greenhouse. Roots were extracted from the dry soil, cut into 5–10 mm pieces and mixed carefully into the soil. This mix of substrate with root segments was used as inoculum of whole rhizosphere microbial communities. The AMF species in the inoculum were identified with spore morphology (Alvarado-Herrejón et al., 2018) (Supplementary Table 2).

2.3. Experimental set-up and conditions

Hybrid maize (DEKALB, 2042) was grown from seed in 1.5 l pots with 1.4 kg sterile soil:sand mix (1:1, w-w) similar to that used for trap cultures of field populations of whole rhizosphere microbial communities. Prior to sowing, mineral fertilizers were mixed into the soil-sand mix similar to the fertilization applied to the trap plants presented above according to the respective treatments with and without N and P. In treatments with P KH_2PO_4 was mixed into the soil prior sowing corresponding to 100 mg P. N was added as NH_4NO_3 each week with 30 mg N per week starting two weeks after sowing until one week before harvest with a total of 180 mg N applied. Inoculum of rhizosphere microorganisms (10% of total pot soil-sand content) were mixed into the soil-sand mix prior sowing in accordance to their respective treatments. Plants were grown under greenhouse conditions for 8 weeks. Watering was performed on a daily basis to maintain 80% of the water holding capacity by weight. Greenhouse temperature was approximately 15 °C at night and 25–30 °C during daytime.

2.4. Harvest and analysis

Plants were harvested 8 weeks after sowing. From each pot the root system was removed and the soil without roots was stored at -20°C until further processing. Roots were detached from shoots and washed and separately oven-dried at 80 °C for 48 h, and weighed.

All dried shoot tissue from each plant were ground and sieved with a mesh No 40 (0.425 mm) before acid digestion and subsequent N and P analyses. Initially, a mixed digester (1 g $CuSO_4$; 10 g K_2SO_4) was added to 0.25 g of shoot dry weight in a 75 mL glass tube. Then 3 mL of H_2O_2 (30% v/v) was added to carry out the oxidation reaction and finally, 7 mL of sulfuric acid (H_2SO_4) was added and left to react for 24 hours. Samples were then placed in a digester block to gradually increase the temperature (50 °C every 20 minutes) to 375 °C for 3 hours. The completely digested mixture was filtered (filter Whatman No 1, 125 mm) and measured by colorimetrical reading at 660 nm in the autoanalyzer Braun + Luebbe III. N was measured with the semi-Kjeldahl method according to Bremner (1996) and P was measured according to Murphy and Riley (1962).

Prior drying, fresh roots was cut in 5–10 mm segments and mixed in water from which a two gram subsample was obtained for measurement of AMF root colonization. Root subsamples were cleared and stained according to Phillips and Hayman (1970) and examined for AMF root colonization using the line-intercept method as described by Giovannetti and Mosse (1980).

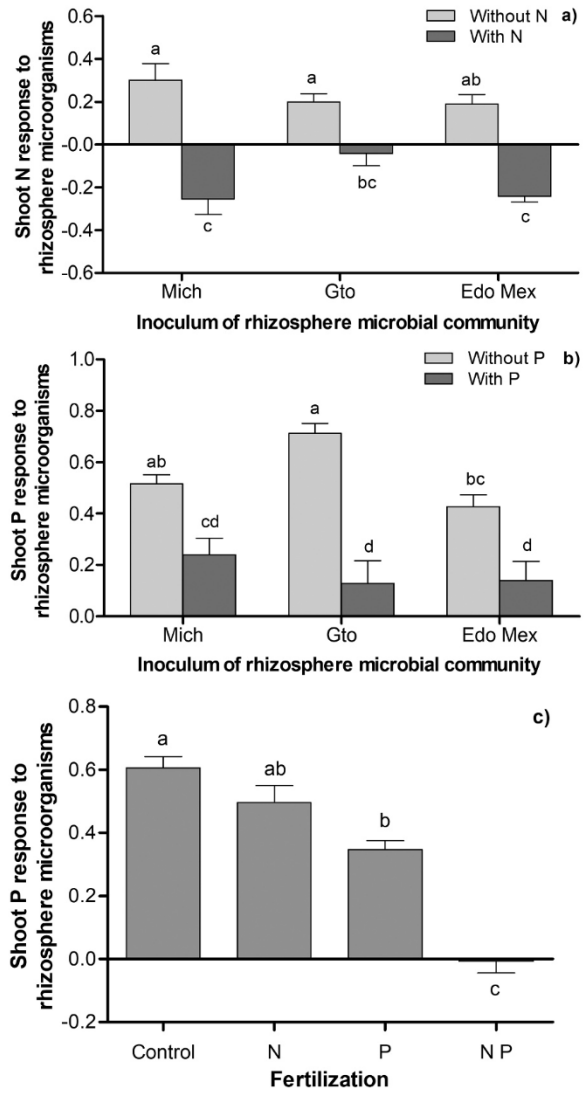

Fig. 3. Shoot N response to rhizosphere microorganisms of the factor treatment means of the Inoculum x N interaction (a) and shoot P response to rhizosphere microorganisms of the factor treatment means of the interactions Inoculum x P (b) and N x P (c) of eight-week-old maize plants. Values represent treatment means with standard error bars (a, $n = 8$; b, $n = 8$; c, $n = 16$). Treatments with different letters are significantly different according to post hoc ANOVA Tukey test ($p \leq 0.05$).

Ten gram of soil samples were freeze dried and powdered in a mortar, of which one-gram subsamples were used for extraction of whole cell fatty acids according to the method of Sasser (1990). To enable quantification of the extracted fatty acids, a known amount of an internal standard, nonadecanoate fatty acid methyl ester 19:0 was added to each sample. Analyses of fatty acid methyl esters were performed using the software package Sherlock Version 6.0 (MIDI Inc.). Hydroxylic (10:0 3OH, 12:0 2OH, 12:0 3OH, 16:0 2OH, 16:0 3OH, 18:0 2OH) and cyclic (17:0 cyclo) fatty acids were used as biomarkers for Gram negative (-) bacteria, fatty acids with branched chains localized on positions iso and anteiso (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0) were used as Gram positive (+) bacteria biomarkers and the methylated fatty acid 17:0 10methyl was used a biomarker for Actinobacteria (Ratledge and Wilkinson, 1988). The fatty acid 16:1ω5 and 18:2ω6,9 were used as biomarkers for AMF (Olsson, 1999) and fungi (Frostegård and Bååth, 1996), respectively. Fatty acid analysis was performed with the Agilent gas chromatograph7890B.

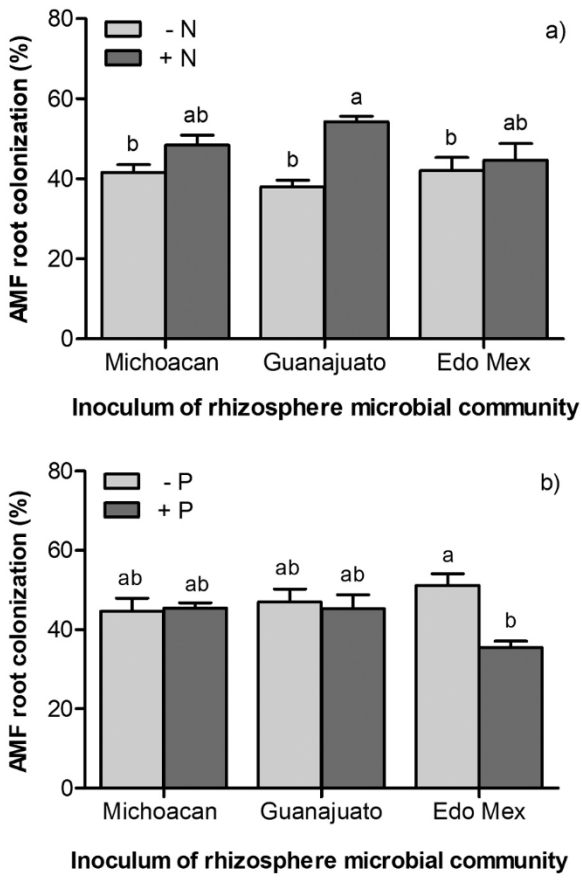

Fig. 4. AMF root colonization of the factor treatment means of Inoculum × N (a) and Inoculum × P (b) interactions of eight-week-old maize plants inoculated with whole rhizosphere microbial communities obtained from Michoacán, Guanajuato and Estado de México. Values represent treatment means with standard error bars (a, $n = 8$; b, $n = 8$). Treatments with different letters are significantly different according to post hoc ANOVA Tukey test ($p \leq 0.05$).

2.5. Statistical analysis

Three way analyses of variance (ANOVA) were performed with the software StatGraphics Centurion XVII and graphs were produced with GraphPad Prism 5. Prior ANOVA variance homogeneity was verified with the Bartlett test. Comparisons between treatment means were performed with post hoc Tukey tests for significant factors and their interactions. Means of AMF root colonization were arcsine transformed prior ANOVA. Power analysis of the obtained statistical results was performed using the software Minitab (Supplementary Table 3).

3. Results

3.1. Shoot and root dry weight

Significant "Inoculum × P × N" interactions were obtained for shoot and root dry weight (Table 1). Combined N and P fertilization markedly increased shoot and root dry weight compared to that without fertilization and with single application of N or P (Fig. 1a,b). Inoculation with the whole rhizosphere microbial communities had no significant effects on shoot and root weight without fertilization and with single application of N or P (Fig. 1a,b). In combination with N and P fertilization the microbial inoculum Michoacán and Estado de Mexico caused marked shoot and root growth suppression, whereas no effect of

Table 2
P values from analysis of variance of the main factors inoculum, N and P fertilization and their interactions for the variables abundance of microbial biomarker fatty acids expressed per unit dry soil or root ($n = 4$).

Microorganisms	Inoculum (I)	P	N	I x P	I x N	P x N	I x P x N
nmole g^{-1} dry soil							
Gram- bacteria	0.3296	0.3789	0.0925	*	0.7896	0.952	0.1565
Gram+ bacteria	0.9972	0.901	0.8413	0.2809	0.7748	0.7237	0.8648
Actinobacteria	*	0.1914	*	0.1129	0.6194	0.9724	0.4743
AMF	***	0.7086	0.8375	*	0.6185	0.7209	0.3917
Fungi	0.123	0.3865	0.4803	0.0714	0.7292	0.7456	0.1304
nmole g^{-1} dry root							
Gram- bacteria	0.6587	0.2346	*	0.0933	0.7203	0.5995	0.1289
Gram+ bacteria	0.2909	***	*	0.2763	0.1332	0.5109	0.6524
Actinobacteria	0.1345	0.6118	0.3728	0.2529	0.8056	0.4853	0.6486
AMF	***	0.0575	**	0.9861	0.1141	0.2514	0.874
Fungi	0.703	0.1579	0.2579	0.6487	0.8033	0.9043	0.177

* $p \leq 0.05$;
** $p < 0.01$;
*** $p < 0.001$.

Guanajuato was observed (Fig. 1a,b).

In terms of total plant growth response to inoculation with whole rhizosphere microbial communities a significant "N x P" interaction was obtained (Table 1). Plant growth promotion was observed with single N fertilization, whereas plant growth suppression was observed without fertilization and with single P fertilization and combined N and P fertilization (Fig. 1c).

3.2. N and P shoot content

Significant "Inoculum × P × N" interactions were obtained for N and P shoot content both in terms of concentration and total amount (Table 1). N fertilization increased shoot N concentration, which however was reduced by inoculation with whole rhizosphere microbial community from Michoacán (Fig. 2a). Inoculation with whole rhizosphere microbial communities increased total shoot N content, but only in combination with single N fertilization (Fig. 2b). Plants fertilized with both N and P had the highest total shoot N content. However, inoculation with the whole rhizosphere microbial communities Michoacán and Guanajuato reduced the total shoot N content (Fig. 2b).

Shoot P concentration was highest with single P fertilization (Fig. 2c). Inoculation with the whole rhizosphere microbial community Guanajuato increased shoot P concentration without fertilization and with single N and P fertilization (Fig. 2c). The two other whole rhizosphere microbial communities Michoacán and Estado de Mexico increased shoot P concentration with single P fertilization (Fig. 2c). On the other hand, no effects of inoculation with the whole rhizosphere microbial communities were observed with combined N and P fertilization (Fig. 2c). Plants fertilized with both N and P had the highest total shoot P content, which however was reduced by inoculation with the whole rhizosphere microbial communities Michoacán and Estado de Mexico (Fig. 2d). In plants with single N fertilization, inoculation with the whole rhizosphere microbial communities Michoacán and Guanajuato increased total shoot P content (Fig. 2d).

A significant "Inoculum × N" interaction was observed for maize shoot N response to inoculation with whole rhizosphere microbial communities (Table 1), which for all communities were positive and negative, without and with N fertilization, respectively (Fig. 3a).

For maize shoot P response to inoculation with whole rhizosphere microbial communities significant "Inoculum × P" and "N × P" interactions were observed (Table 1). Positive shoot P response was observed for all three whole rhizosphere microbial communities both with and without P fertilization, but with stronger response without P fertilization (Fig. 3b). On the other hand combined N and P fertilization resulted in a slight negative shoot P response to inoculation with whole rhizosphere microbial communities (Fig. 3c).

3.3. AMF root colonization

Significant "Inoculum × N" and "Inoculum × P" interactions were observed for AMF root colonization (Table 1). N fertilization increased AMF root colonization from the Guanajuato whole rhizosphere microbial community, but had no effect on the two other communities (Fig. 4a). P fertilization reduced AMF root colonization from the Estado de Mexico whole rhizosphere microbial community, but had no effect on the two other communities (Fig. 4b).

3.4. Abundance of rhizosphere microorganisms

A significant "Inoculum × P" interaction was observed for the abundance of Gram- bacteria (Table 2), however after the post-hoc ANOVA factor treatment means comparison no significant differences were observed. For the abundance of AMF a single Inoculum factor effect and an "Inoculum × P" interaction was observed (Table 2). In general the abundance of AMF was higher in treatments with inoculation of rhizosphere microorganisms (Fig. 5a). For Actinobacteria significant single factor effects of "Inoculum" and "N" were observed (Table 1), with higher abundance in inoculum from Michoacán (Fig. 5b) and in treatments with N fertilization (Fig. 5c).

For root specific abundance of rhizosphere microorganisms a significant effect of Inoculum was observed for the variable AMF (Table 2), where treatments with inoculation with rhizosphere microbial communities had higher values than the non-inoculated control (Fig. 6d). Significant single factor effects of "N" were observed for the root specific abundance of biomarker fatty acids Gram+ bacteria, Gram- bacteria and AMF (Table 2), which in all cases were reduced by N fertilization (Fig. 6a,c,e). Also a significant single factor "P" effect was observed for the root specific abundance of Gram+ bacteria (Table 1), where P fertilization reduced this variable (Fig. 6b).

3.5. Correlation analysis

From the correlation analysis linking plant and microbial parameters significant positive correlations were observed for AMF root colonization with shoot N concentration, total shoot N content and plant growth response to whole rhizosphere microbial communities (Table 3). Regarding abundance of rhizosphere microorganisms significant positive correlations were observed for Actinobacteria with

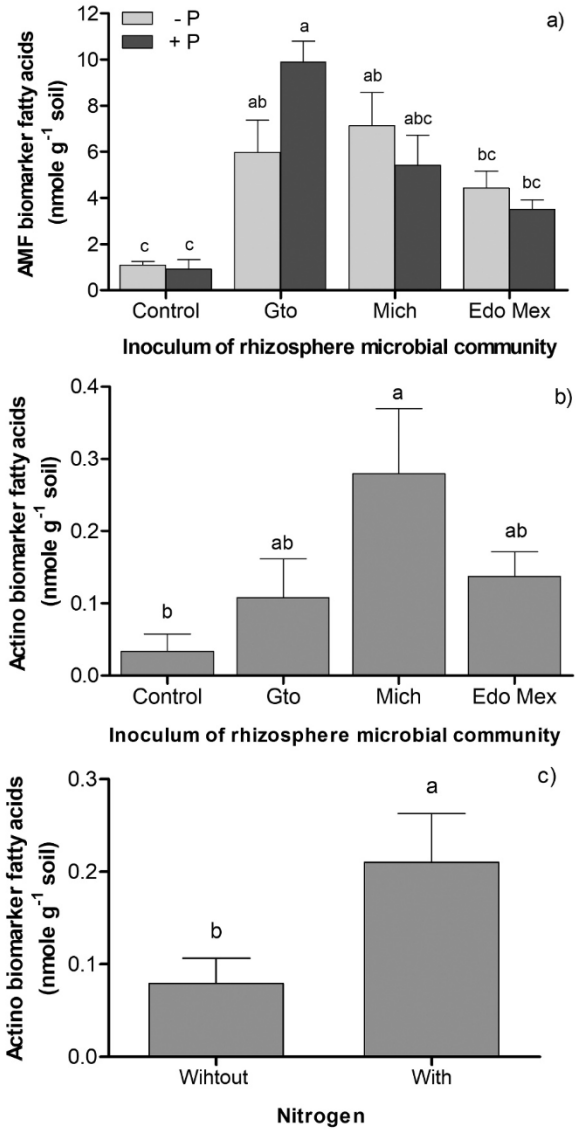

Fig. 5. Factor treatment means of the "Inoculum × P" interaction of the abundance of the AMF (nmole g^{-1} soil) (a) and single factor effects of "Inoculum" (b) and N (c) of the abundance of Actinobacteria (nmole g^{-1} soil) of eight-week-old maize plants. Values represent treatment means with standard error bars (a, $n = 8$; b, $n = 16$; c, $n = 32$). Treatments with different letters are significantly different according to post hoc ANOVA Tukey test ($p \leq 0.05$).

shoot N concentration and shoot N response to whole rhizosphere microbial communities (Table 3).

Multiple correlations were observed between root specific abundance of rhizosphere microorganisms and plant parameters (Table 3). For shoot dry weight only negative correlation was observed with specific abundance of Gram+ bacteria, whereas for root dry weight root specific abundance of all microbial groups except Actinobacteria correlated negatively (Table 3). Abundance of root specific Actinobacteria correlated positively with shoot N concentration and shoot N response to rhizosphere microorganisms, but correlated negatively with plant growth response to rhizosphere microorganisms (Table 3). Abundance of root specific abundance of Gram+ bacteria correlated negatively with total shoot P and N (Table 3). For shoot P response to rhizosphere microorganisms positive correlations were observed with all root specific abundance of all groups of microorganisms except Actinobacteria (Table 3).

3.6. Statistical power analysis

In general the power analysis for all three factors of the ANOVA revealed high statistical power (> 80%) for all plant variables, whereas lower statistical power was obtained for the abundance of rhizosphere microorganisms (Supplementary material Table 3). In most cases significant effects coincided with high statistical power.

4. Discussion

Here we show that mineral N and P fertilization strongly affect the contribution of whole rhizosphere microbial communities to maize growth and nutrition. Our hypothesis that maize plant growth promotion and nutrition by whole rhizosphere microbial communities is higher under N and P nutrient limitations was only partially confirmed. Instead our results show that maize plant growth and nutrient response to whole rhizosphere microbial communities depends on the balance between N and P fertilization and that N limitation result in plant growth suppression.

The observed effects of whole rhizosphere microbial communities on maize plant growth seem to be ascribed mainly to AMF and Actinobacteria as indicated from the positive and negative correlation with maize plant growth response, respectively. Plant growth promotion was obtained only in P limited soil without N limitation, which is a common response of maize to inoculation with single species (Sawers et al., 2017) and field populations (Sarabia et al., 2017; Zitlalpopoca-Hernandez et al., 2017) of AMF. On the contrary rhizosphere microorganisms caused plant growth suppression when maize plants were grown under N limited conditions. Similar results were reported by Johnson et al. (2015) showing that AMF field populations can ameliorate P limitation, but not N limitation in the grass *Andropogon gerardii*. Indeed it is well established that AMF can promote P nutrition of their host and to a limited extend also N nutrition (Smith and Smith, 2011).

Two out of the three rhizosphere microbial communities caused strong plant growth suppression in maize when grown in soil without nutrient limitations, which is most likely the result of a parasitic maize-AMF association, where the cost of hosting the AMF in terms of photosynthates was higher than the benefit in terms of P acquisition. Similar results were reported by Sarabia et al. (2017) showing that a field population of AMF caused strong plant growth suppression in maize grown in soil with mineral P fertilization. Plant growth depression is a common mycorrhizal phenotype mainly caused by low light intensity and high P fertilization (Johnson and Graham, 2013). However, from the observed negative correlation between abundance of Actinobacteria and plant growth response to whole rhizosphere microbial communities, it is important to further address the role of Actinobacteria in plant growth suppressions, which may not only be a question of carbon drain.

Our results showing that the percentage of AMF root colonization correlated positively with both shoot N concentration and total shoot N content, underlines the importance of AMF in plant and soil N dynamics, which however is an area of AMF research with a substantial knowledge gap (Larsen et al., 2015).

Mineral phosphorus fertilization is known to decrease root colonization of field populations of AMF (Sarabia et al., 2017; Toljander et al., 2008; Johnson et al., 2006; Thomson et al., 1992), which in the present study was only observed for the rhizosphere microbial community from Estado de Mexico. On the contrary AMF root colonization with the rhizosphere microbial community from Guanajuato was increased by N fertilization. Similar findings were reported by Aguilar et al. (2017) showing that NPK fertilization increased AMF root colonization of native field populations of AMF. No clear response pattern of AMF root colonization to mineral fertilization scenarios can be obtained from the

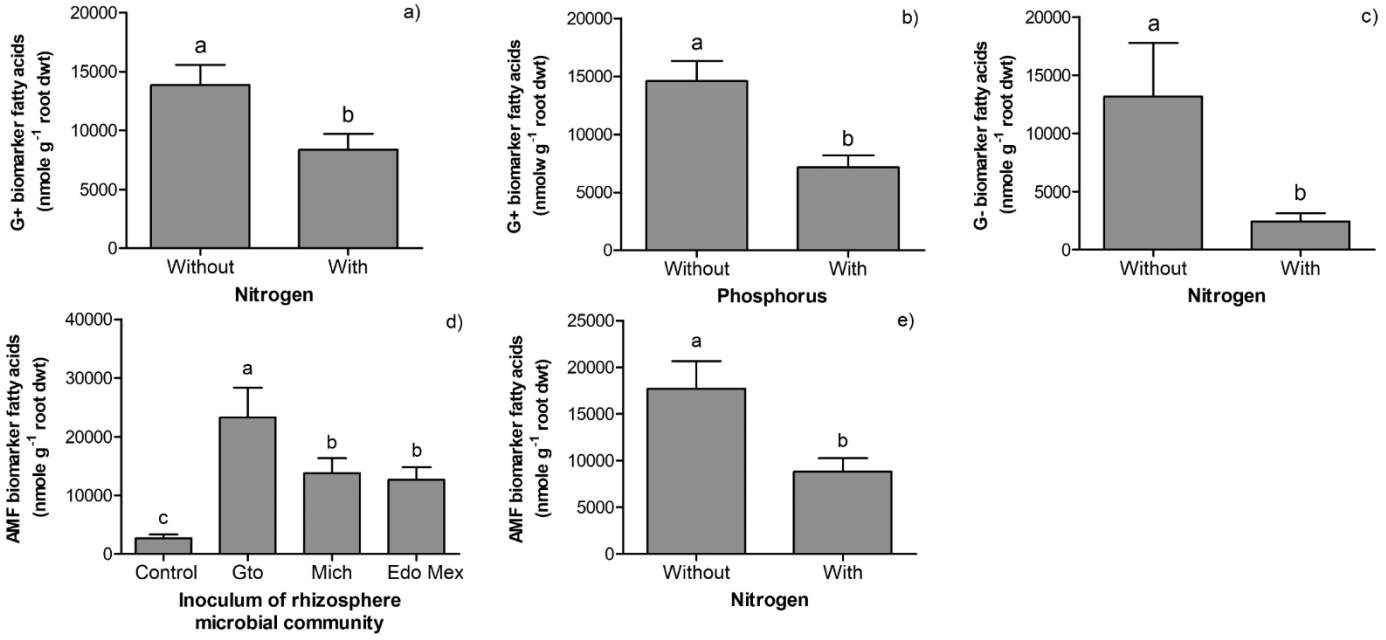

Fig. 6. Factor treatment means of single factor effects of N on the root specific abundance (nmole g^{-1} root) of Gram + bacteria (a), Gram- bacteria (c) and AMF (e), single factor effect of P on the root specific abundance of Gram- bacteria (b) and single factor effect of Inoculum on the root specific abundance of AMF (d) of eight-week-old maize plants. Values represent treatment means with standard error bars (a, $n = 32$; b, $n = 32$; c, $n = 32$; d, $n = 16$; e, $n = 32$). Treatments with different letters are significantly different according to post hoc ANOVA Tukey test ($p \leq 0.05$).

present study, but our results suggest a possible adaptation of field populations of AMF to agricultural practice such as mineral fertilization in the environment of their origin. Interestingly, the AMF in the rhizosphere microbial community from Estado de Mexico and Guanajuato have been exposed to high and low input of mineral fertilizer inputs, respectively, which may have resulted in some level of environmental adaptation of the native AMF. However, this needs to be further addressed with an experimental design exploring this question in particular.

This evident strong complexity of AMF response to mineral N and P fertilization was further underlined with the observed limited effects on AMF soil colonization as measured in terms of the abundance of the AMF biomarker fatty acid 16:1ω5. On the contrary, other studies have reported reduction in AMF soil colonization both from native AMF communities (Gryndler et al. 2006) and single species (Olsson et al. 1995).

Plant growth and nutrient promotion from root associated bacteria including *Azospirillum*, *Pseudomonas*, *Burkholderia* and *Bacillus,* which are common and abundant inhabitants of the rhizosphere microbiome, have been well studied (Rosenblueth and Martínez-Romero, 2006; Dobbelaere et al., 2003). In the present study, whole rhizosphere microbial communities could not ameliorate maize N deficiency in terms of plant growth confirming the general consensus that N fixation from non-symbiotic diazotrophic bacteria in agroecosystems is limited (Hodge and Storer, 2014; Herridge et al., 2008).

Overall our results suggest a positive contribution of whole rhizosphere microbial communities for maize shoot P independent of N fertilization though higher without P fertilization. Indeed the plant P uptake from the mycorrhiza pathway has been shown to be important independent of mycorrhiza plant growth response and P soil availability (Smith et al., 2003). On the other hand, our results also suggest that the abundance of Actinobacteria in the rhizosphere soil and as well as root specific abundance associate with maize shoot N content and shoot N response to whole rhizosphere microbial communities, which indicate that Actinobacteria are involved in maize N nutrition.

The observed positive correlation between shoot P response and root specific abundance of all groups of rhizosphere microorganisms except Actinobacteria, underlines the importance of rhizosphere microorganisms assisted plant P uptake, which is in accordance to Richardson et al. (2011). On the other hand, in terms of root specific abundance only Actinobacteria correlated positively with shoot N response, which suggest that for maize N nutrition the rhizosphere assisted N uptake is of less importance. Also it is important to note that the root specific abundance of Actinobacteria correlated negatively with plant growth response to whole rhizosphere microbial communities, which may have resulted in increased shoot N concentration.

On the other hand the contribution of whole rhizosphere microbial communities for maize shoot N content was positive and negative without and with N fertilization respectively, suggesting competition between roots and rhizosphere microorganisms for N, when N is limited for plant growth, which is in accordance with Kuzyakov and Xu (2013).

In general effects of N and P fertilization on the abundance of rhizosphere microorganisms were limited when expressed per g soil, but when expressed per gram root dry weight in terms of root specific abundance N fertilization reduced the amount of Gram- and Gram + bacteria as well as AMF, whereas P fertilization only reduced the amount of Gram + bacteria. These findings are in accordance with (Carvalhais et al. 2011) showing that N and P fertilization alter the composition and reduce the amount of root exudates and consequently the abundance and community composition of microorganisms thriving from these exudates.

Plant growth and nutrition promoting by rhizosphere microorganism, including AMF and rhizobacteria, offer several key ecosystem services in agroecosystems in relation to plant and soil nutrient dynamics (Larsen et al., 2015), which should be conserved and managed as a central component to improve nutrient use efficiency (Richardson et al., 2009) and soil fertility management (Drinkwater and Snapp, 2007). Here we show that mineral N and P fertilization strongly associate with the contribution of whole rhizosphere microbial communities in maize growth and nutrition. However, response of rhizosphere microorganism to other agricultural practices such as crop genotype, crop rotation, tillage, crop protection measures and organic fertilization should be further addressed to fully explore the benefits of rhizosphere microorganisms in agroecosystems (Larsen et al., 2014).

Table 3
Pearson correlation analysis between plant and microbial variables measured at harvest of eight-week-old maize plants with all 64 experimental units.

	Shoot dry weight		Root dry Weight		Shoot P concentration		Shoot N concentration		Total shoot P		Total shoot N		Plant growth response		Shoot P response		Shoot N response	
	p	*r*	*P*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*R*	*p*	*r*	*p*	*R*	*p*	*r*
AMF root colonization	0.2767	0.2368	0.5484	0.1319	0.089	-0.3626	**	0.5636	0.3648	0.1981	*	0.4172	*	0.4504	0.6908	0.0877	0.3641	-0.1984
Abundance of rhizosphere microorganisms (nmole g^{-1} dry soil)																		
Gram-	0.1707	-0.2957	0.123	-0.3309	0.9494	-0.014	0.1327	-0.323	0.1539	-0.3072	0.1232	-0.3308	0.7606	-0.0672	0.0677	0.3875	0.0886	-0.3631
Gram +	0.6504	-0.0998	0.4873	-0.1525	0.3509	-0.2038	0.8839	0.0322	0.5166	-0.1425	0.7331	-0.0752	0.6074	-0.1131	0.5875	0.1194	0.4368	-0.1704
Actinobacteria	0.2448	0.2526	0.3327	0.2115	0.2843	-0.2332	*	0.481	0.1207	0.3328	0.0946	0.3569	0.1137	-0.3389	0.1008	-0.3507	*	0.4256
AMF	0.2459	0.2521	0.3963	0.1857	0.4071	-0.1815	0.286	0.2324	0.3293	0.2129	0.1716	0.2951	0.236	0.2573	0.9401	-0.0166	0.5498	-0.1315
Fungi	0.3169	-0.2183	0.2145	-0.269	0.9559	0.0122	0.2627	-0.2436	0.3215	-0.2163	0.2739	-0.2381	0.8536	-0.0407	0.0864	0.3654	0.2489	-0.2505
Root specific abundance of rhizosphere microorganisms (nmole g^{-1} dry root)																		
Gram-	0.0736	−0.3802	*	−0.4233	0.9472	0.0146	0.1422	−0.3158	0.0593	−0.3989	0.0612	−0.3963	0.7379	−0.0738	*	0.4694	0.072	−0.3821
Gram +	**	−0.5241	**	−0.5926	0.8546	0.0405	0.4314	−0.1725	**	−0.5491	*	−0.4886	0.6159	−0.1104	**	0.5378	0.1832	−0.2877
Actinobacteria	0.7118	−0.0814	0.5637	−0.127	0.6924	−0.0872	*	0.4408	0.8773	0.0341	0.7503	0.0702	*	−0.4326	0.5471	−0.1324	*	0.4363
AMF	0.0742	−0.3794	*	−0.4686	0.7377	0.0739	0.9564	−0.0121	0.0555	−0.4046	0.1366	−0.32	0.6657	0.0952	*	0.4999	0.2399	−0.2552
Fungi	0.092	−0.3595	*	−0.4189	0.7913	0.0584	0.2273	−0.2619	0.0935	−0.358	0.0903	−0.3613	0.7854	−0.0601	*	0.4964	0.1703	−0.296

*** $p < 0.001$.
* $p \leq 0.05$.
** $p < 0.01$.

In conclusion, our results show that whole rhizosphere microbial communities can promote maize plant growth and nutrition, which however strongly depends on the balance between N and P fertilization. In addition, microbial assisted nutrient uptake in maize seems to be more important for P than for N.

Acknowledgments

We thank the postgraduate biological sciences program of the National Autonomous University of México for facilitating doctorate biological science training for the first author of this article. We also thank the National Council for Science and Technology (CONACYT) for funding the basic science project 179319. Finally we extend our gratitude to Maribel Nava Mendoza for excellent technical support for P and N measurements.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rhisph.2018.11.004.

References

Aguilar, R., Carreón-Abud, Y., López-Carmona, D., Larsen, J., 2017. Organic fertilizers alter the composition of pathogens and arbuscular mycorrhizal fungi in maize roots. J. Phytopathol. 165, 448–454. https://doi.org/10.1111/jph.12579.

Alvarado-Herrejón, M., Larsen, J., Gavito, M.E., Jaramillo-López, P.F., Vestberg, M., Martínez-Trujillo, M., Carreón-Abud, Y., 2018. Relation between arbuscular mycorrhizal fungi, root-lesion nematodes and soil characteristics in maize agroecosystems. Appl. Soil Ecol. https://doi.org/10.1016/j.apsoil.2018.10.019. (In press).

Berta, G., Copertta, A., Gamalero, E., Bona, E., Cesaro, P., Scarafoni, A., D'Agostino, G., 2013. Maize development and grain quality are differentially affected by mycorrhizal fungi and a growth-promoting pseudomonad in the field. Mycorrhiza 24, 161–170.

Bhadalung, N., Suwanarit, A., Dell, B., Nopamornbodi, O., Thamchaipenet, A., Rungchuang, J., 2005. Effects of long-term NP-fertilization on abundance and diversity of arbuscular mycorrhizal fungi under a maize cropping system. Plant Soil 270, 371–382. https://doi.org/10.1007/s11104-004-1829-4.

Bremner, J., 1996. Nitrogen - total. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R., Soltanpour, P., Tabatabai, M., Johnston, C.T., Sumner, M.E. (Eds.), Nitrogen-total in Methods Of Soil Analysis Part 3 - Chemical Methods, Madison, pp. 1085–1121.

Carvalhais, L.C., Dennis, P.G., Fedoseyenko, D., Hajirezaei, M.R., Borriss, R., et al., 2011. Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. J. Plant Nutr. Soil Sci. 174, 3–11.

Dobbelaere, S., Vanderleyden, J., Okon, Y., 2003. Plant growth-promoting effects of diazotrophs in the rhizosphere. CRC Crit. Rev. Plant Sci. 22, 107–149. https://doi.org/10.1080/713610853.

Drinkwater, L.E., Snapp, S.S., 2007. Nutrients in agroecosystems: rethinking the management paradigm. Adv. Agron. 92, 163–186. https://doi.org/10.1016/S0065-2113(04)92003-2.

Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–65. https://doi.org/10.1007/BF00384433.

Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x.

Gryndler, M., Larsen, J., Hršelová, H., Řezáčová, V., Gryndlerová, H., Kubát, J., 2006. Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. Mycorrhiza 16, 159–166. https://doi.org/10.1007/s00572-005-0027-4.

Gutiérrez-Zamora, M.L., Martínez-Romero, E., 2001. Natural endophytic association between Rhizobium etli and maize (Zea mays L.). J. Biotechnol. 91, 117–126. https://doi.org/10.1016/S0168-1656(01)00332-7.

Harman, G.E., Petzoldt, R., Comis, A., Chen, J., 2004. Interactions between Trichoderma harzianum strain T22 and maize inbred line Mo17 and effects of these interactions on diseases caused by Pythium ultimum and Colletotrichum graminicola. Phytopathology 94, 147–153.

Herridge, D.F., Peoples, M.B., Boddey, R.M., 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311, 1–18. https://doi.org/10.1007/s11104-008-9668-3.

Hodge, A., Storer, K., 2014. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant Soil 386, 1–19. https://doi.org/10.1007/s11104-014-2162-1.

Johnson, N.C., Graham, J.H., 2013. The continuum concept remains a useful framework for studying mycorrhizal functioning. Plant Soil 363, 411–419. https://doi.org/10.1007/s11104-012-1406-1.

Johnson, N.C., Hoeksema, J.D., Bever, J.D., Chaudhary, V.B., Gehring, C., Klironomos, J.N., Koide, R.T., Miller, R.M., Moore, J., Moutoglis, P., Schwartz, M., Simard, S., Swenson, W., Umbanhowar, J., Wilson, G., Zabinski, C., 2006. From Lilliput to

Brobdingnag: extending models of mycorrhizal function across scales. Bioscience 56, 889–900. https://doi.org/10.1641/0006-3568(2006)56[889:FLTBEM]2.0.CO;2.
Johnson, N.C., Wilson, G.W.T., Wilson, J.A., Miller, R.M., Bowker, M.A., 2015. Mycorrhizal phenotypes and the law of the minimum. New Phytol. 205, 1473–1484. https://doi.org/10.1111/nph.13172.
Kuzyakov, Y., Xu, X., 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol. 198, 656–669.
Larsen, J., Jaramillo-Lopez, P., Najera-Rincon, M., Gonzalez-Esquivel, C.E., 2015. Biotic interactions in the rhizosphere in relation to plant and soil nutrient dynamics. J. Soil Sci. Plant Nutr. 15, 449–463. https://doi.org/10.4067/S0718-95162015005000039.
Larsen, J., Nájera-Rincon, M., Esquivel, C., Gavito, M., 2014. Management of rhizosphere microorganisms in relation to plant nutrition and health. In: Agroecology, Ecosystems, and Sustainability. Informa UK. doi: ⟨https://doi.org/103-120103-120⟩, ⟨http://dx.doi.org/10.1201/b17775-6⟩.
Larsen, J., Pineda-Sánchez, H., Delgado-Arellano, I., Castellano-Morales, V., Carreto-Montoya, L., Villegas-Moreno, J., 2017. Interactions between microbial plant growth promoters and their effects on maize growth performance in different mineral and organic fertilization scenarios. Rhizosphere 3, 75–81. https://doi.org/10.1016/j.rhisph.2017.01.003.
Leggett, M., Newlands, N.K., Greenshields, D., West, L., Inman, S., Koivunen, M.E., 2015. Maize yield response to a phosphorus-solubilizing microbial inoculant in field trials. J. Agric. Sci. 153, 1464–1478.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.
Olsson, P.A., Bååth, E., Jakobsen, I., Söderström, B., 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycol. Res. 99, 623–629. https://doi.org/10.1016/S0953-7562(09)80723-5.
Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interaction of mycorrhizal fungi in soil. FEMS Microbiol. Ecol. 29, 303–310.
Peiffer, J.A., Spor, A., Koren, O., Jin, Z., Tringe, S.G., Dangl, J.L., Buckler, E.S., Ley, R.E., 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc. Natl. Acad. Sci. USA 110, 6548–6553.
Philippot, L., Raaijmakers, J., Lemanceau, P., van der Putten, W., 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789–799.
Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–160. https://doi.org/10.1016/S0007-1536(70)80110-3.
Ranum, P., Peña-Rosas, J.P., Garcia-Casal, M.N., 2014. Global maize production, utilization, and consumption. Ann. N.Y. Acad. Sci. 1312, 105–112. https://doi.org/10.1111/nyas.12396.
Ratledge, C., Wilkinson, S., G., 1988. Microbial Lipids, 1st ed. Academic Press, London.
Richardson, A.E., Barea, J.M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321, 305–339. https://doi.org/10.1007/s11104-009-9895-2.
Richardson, A., Lynch, J., Ryan, P., Delhaize, E., Smith, F., Smith, S., Harvey, P., Ryan, M., Veneklaas, E., Lambers, H., Oberson, A., Culvenor, R., Simpson, R., 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant Soil 349, 121–156.
Rosenblueth, M., Martínez-Romero, E., 2006. Bacterial endophytes and their interactions with hosts. Mol. Plant-Microbe Interact. 19, 827–837. https://doi.org/10.1094/MPMI-19-0827.
Sarabia, M., Cornejo, P., Azcón, R., Carreón-Abud, Y., Larsen, J., 2017. Mineral phosphorus fertilization modulates interactions between maize, rhizosphere yeasts and arbuscular mycorrhizal fungi. Rhizosphere 4, 89–93. https://doi.org/10.1016/j.rhisph.2017.09.001.
Sarabia, M., Cazares, S., González-Rodríguez, A., Mora, F., Carreón-Abud, Y., Larsen, J., 2018. Plant growth promotion traits of rhizosphere yeasts and their response to soil characteristics and crop cycle in maize agroecosystems. Rhizosphere 6, 67–73.
Sasser, M., 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI technical note 101. MIDI, Newark, DE19711, USA.
Sawers, R.J.H., Svane, S.F., Quan, C., Grønlund, M., Wozniak, B., Gebreselassie, M.N., González-Muñoz, E., Chávez-Montes, R.A., Baxter, I., Goudet, J., Jakobsen, I., Paszkowski, U., 2017. Phosphorus acquisition efficiency in arbuscular mycorrhizal maize is correlated with the abundance of root-external hyphae and the accumulation of transcripts encoding PHT1 phosphate transporters. New Phytol. 214, 632–643. https://doi.org/10.1111/nph.14403.
Smith, S.E., Smith, F.A., Jakobsen, I., 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol. 133, 16–20.
Smith, S.E., Smith, F.A., 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Ann. Rev. Plant Biol. 62, 227–250. https://doi.org/10.1146/annurev-arplant-042110-103846.
Thomson, B.D., Robson, A.D., Abbott, L.K., 1992. The effect of long-term applications of phosphorus fertilizer on populations of vesicular-arbuscular mycorrhizal fungi in pastures. Aust. J. Agric. Res. 43, 1131–1142. https://doi.org/10.1071/AR.(9921131).
Toljander, J.F., Santos-González, J.C., Tehler, A., Finlay, R.D., 2008. Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial. FEMS Microb. Ecol. 65, 323–338. https://doi.org/10.1111/j.1574-6941.2008.00512.x.
Treseder, K.K., Allen, M.F., 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. New Phytol. 155, 507–515. https://doi.org/10.1046/j.1469-8137.2002.00470.x.
Zitlalpopoca-Hernandez, G., Najera-Rincon, M.B., Del-Val, E., Alarcon, A., Jackson, T., Larsen, J., 2017. Multitrophic interactions between maize mycorrhizas, the root feeding insect Phyllophaga vetula and the entomopathogenic fungus Beauveria bassiana. Appl. Soil Ecol. 115, 38–43. https://doi.org/10.1016/j.apsoil.2017.03.014.
Vázquez, M., César, S., Azcón, R., Barea, J.M., 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (Azospirillum, Pseudomonas, Trichoderma) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl. Soil Ecol. 15, 261–272.
Walters, W.A., Jin, Z., Youngblut, N., Wallace, J.G., Sutter, J., Zhang, W., González-Peña, A., Peiffer, J., Koren, O., Shi, Q., Knight, R., Glavina del Rio, T., Zringe, S.G., Buckler, E.S., Dangl, J.J., Ley, R.E., 2018. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proc. Natl. Acad. Sci. USA 115, 7368–7373.