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Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities

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ABSTRACT

We studied the microbial communities in maize (*Zea mays*) rhizosphere to determine the extent to which their structure, biomass, activity and growth were influenced by plant genotype (*su1* and *sh2* genes) and the addition of standard and high doses of different types of fertilizer (inorganic, raw manure and vermicompost). For this purpose, we sampled the rhizosphere of maize plants at harvest, and analyzed the microbial community structure (PLFA analysis) and activity (basal respiration and bacterial and fungal growth rates). Discriminant analysis clearly differentiated rhizosphere microbial communities in relation to plant genotype. Although microorganisms clearly responded to dose of fertilization, the three fertilizers also contributed to differentiate rhizosphere microbial communities. Moreover, larger plants did not promoted higher biomass or microbial growth rates suggesting complex interactions between plants and fertilizers, probably as a result of the different performance of plant genotypes within fertilizer treatments, i.e. differences in the quality and/or composition of root exudates.

1. Introduction

The rhizosphere is the biologically active zone of soil where plant roots and microorganisms interact, and is of great importance for plant performance as well as for nutrient cycling and ecosystem functioning (Singh et al., 2004). The interactions involve root exudates, which shape the structure and enhance the activity of microbial communities, and the nutrients released by microorganisms, which affect plant growth (Paterson et al., 2007). Root exudates comprise a wide range of substances including sugars, amino acids, siderophores and enzymes (reviewed in Uren, 2007). Moreover, root exudates, which largely determine the composition of the rhizosphere microbial community and the soil microbial pool, depend among other factors on plant species, genotype and fertilization regime (Appuhn and Joergensen, 2006; Garbeva et al., 2004; Rengel and Marschner, 2005; Smith et al., 1999). For example, maize hybrids are able to select specific bacterial strains that their parents do not, thereby increasing the diversity of rhizosphere bacteria (Picard and Bosco, 2005, 2006; but see Roesch et al., 2006). Although long-term application of large amounts of inorganic fertilizers may enhance plant yield, it may also reduce microbial diversity, biomass and activity (Johnson et al., 2005; Mäder et al., 2002). However, such negative effects on soil microorganisms may be avoided by the application of organic fertilizers (Edwards, 2004; Esperschütz et al., 2007; Hartmann et al., 2006; Toljander et al., 2008). Fertilization influences plant physiological status, as high nutrient availability favours the release of root exudates, whereas low nutrient availability restricts the allocation of plant resources to root exudates (Neumann and Römheld, 2007).

The structure of rhizosphere microbial communities is the result of complex interactions between plant genotype and fertilization. Root exudates from maize are composed of 65% sugars, 33% organic acids and 2% amino acids and fertilization modifies the composition of root exudates, leading to increased bacterial biomass and different bacterial community structure (Baudoin et al., 2003).

The objective of the present study was to investigate how plant genotype and fertilization regime influence the structure and activity of rhizosphere microbial communities. We tested the effects of plant genotype because it is known that different maize plant genotypes produce different root exudates (Corrales et al., 2007). We selected cultivars of maize with the sugary endosperm mutation (*su1*) and with the shrunken endosperm mutation (*sh2*), which differ in their C storage patterns. The *sh2* plants store C as starch and *su1* plants as a diverse array of polysaccharides, and this should be reflected in the composition of root exudates (Revilla et al., 2006). Thus, we hypothesized that the different C allocation

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strategies in the different plant genotypes should promote different rhizosphere microbial communities. We studied two organic amendments, raw manure and vermicompost, because they differ in their chemical and microbial characteristics (Edwards, 2004; Domínguez et al., 2010). We used standard and high doses of the fertilizers because plant physiological status will depend on nutrient availability, which would indirectly affect rhizosphere microorganisms. Furthermore, annual plants like maize reduce or cease root exudation when all plant resources are allocated to fructification (Taiz and Zeiger, 2006), and rhizosphere microbial communities depend on soil nutrient pools (Brimecombe et al., 2007). Consequently, the amount of nutrients that remains unused in the soil increases with the dose of fertilizer. We also hypothesized that in the absence of any effects of plant genotype, the structure and abundance of rhizosphere microbial communities would differ in inorganic and organic treatments, and that the effect would be dose-dependent.

2. Materials and methods

2.1. Site, substrates and plants

The experiment was set up in an experimental site (Misión Biolóxica de Galicia, Pontevedra, Spain: 42°24′N, 8°38′W). The mean temperature during the growing season (May–October) was 18.9 ± 0.8 °C, with a maximum of 25.6 °C and a minimum of 11.4 °C; the mean precipitation during the study period was 70.1 ± 20.7 mm.

We used maize *Zea mays* L. since it is homozygous for one or more genes that multiply the levels of sugar in the endosperm (Revilla and Tracy, 1995). Here we used two maize hybrids developed by crossing: I453x101t and P39xC23, which differ in two genes that modify the sugar content of the endosperm, *sugary1* (*su1*) located on the short arm of chromosome 4, and *shrunken2* (*sh2*) located on the long arm of chromosome 3. The types and amounts of grain polysaccharides are modified in plants with *su1*, whereas plants with *sh2* store sugars instead of starch (Revilla et al., 2006).

2.2. Experimental design

The experiment was arranged as a randomized block design, with separate blocks for *su1* and *sh2*, since maize hybrids with different endosperm type must be tested in different blocks to avoid the effects of xenia (the direct effect of pollen on the phenotype of the kernel). These plant genotypes should be isolated from one another, either by distance or by adequate borders consisting of the appropriate endosperm type (Tracy, 2001). Blocks of *sh2* and *su1* plants were separated by a row of shrunken (EPS18) and sugary (Golden Bantam) next to *sh2* and *su1* plants, respectively. Two blocks were established per plant gene (*su1* and *sh2*). In each block all possible combinations of experimental factors (genetic background, and type and rate of fertilization) were established in individual plots. Each experimental plot (10 m^2) included two rows spaced 0.8 m apart, with 25 two-plant hills spaced 0.21 m apart. Plots were overplanted and thinned to obtain a mean density of 60,000 plants per ha.

Organic and inorganic (NPK) fertilizers were used; the organic fertilizers were raw rabbit manure and vermicompost, the latter obtained from the raw rabbit manure. The fertilizers were applied at two different doses: standard (80:24:20 kg NPK ha^{-1}) and high (120:36:30 kg NPK ha^{-1}) to achieve a mean production of 4 and 6 T dry grain per ha, respectively. The organic treatments provided only 25% of the nutrient requirements for maize growth and the remaining 75% was supplied as inorganic fertilizer. Fertilizers were spread on the soil and incorporated by mixing into the first 20 cm of the soil. The dose of fertilizer (inorganic and organic) added was calculated by taking into account the nutrient content (NPK) of the soil and fertilizers. Thus, the standard dose was 5.4 and 4.2 T substrate per ha for manure and vermicompost, respectively, and the high dose was 8.2 and 6.3 T substrate per ha for manure and vermicompost, respectively. As a consequence of the nutrient content of the substrates, the plots treated with organic fertilizers received a surplus of C in relation to the plots treated with inorganic fertilizers. The C surplus was 0.24 and 1.6 kg C m^{-2} in the manure treatments and 0.9 and 1.4 kg C m^{-2} in the vermicompost treatments, for standard and high doses of fertilizer, respectively. In all treatments, 60% of the total N was supplied at sowing while the remaining 40% was provided as top dressing during stalk formation of plants. The plots were sown in mid May and were weeded manually through the experiment.

At harvest (late August), five plants were selected from each plot, the shoots clipped and the roots unearthed with a fork. Rhizosphere soil was sampled in two steps. Firstly, the root system was separated from the bulk soil by gentle shaking, followed by more vigorous shaking; soil still adhering to the roots was considered as rhizosphere soil, and was removed from roots by further shaking. Composite samples, consisting of subsamples from five plants, were placed in labelled plastic bags and sieved (2 mm) prior to analysis.

2.3. Analytical methods

Microbial communities were assessed by phospholipid fatty acid (PLFA) analysis (Gómez Brandón et al., 2010). Total lipids were extracted from 2 g (dry weight) of soil samples with methanol and chloroform (1:2 v:v). The mixture was then filtered and evaporated under a stream of N_2 gas. The total lipid extract was then dissolved with chloroform (3 × 1 mL). Lipids were separated into neutral, glycol- and phospholipids on silicic acid columns (Strata SI-1 Silica (55 μm, 70 Å), 500 mg/6 mL) with chloroform, acetone and methanol. The fraction containing phospholipids was evaporated under a N_2 stream and re-dissolved in 500 μL of methyl-*tert*-butyl ether. One hundred microliters of this solution were placed in a 1.5 mL vial with 50 μL of the derivatizating agent (trimethylsulfonium hydroxide, TMSH), vortexed for 30 s and allowed to react for 30 min; 10 μL of nonadecanoic acid methyl ester were then added as an internal standard. The chromatographic conditions are described elsewhere (Gómez Brandón et al., 2010). In order to identify and quantify the fatty acids, retention times and mass spectra were compared against those obtained for known standard mixtures or pure PLFAs.

The PLFAs used as biomarkers were as defined in the literature (Bååth, 2003; Frostegård and Bååth, 1996). Total living microbial biomass was determined as the sum of all extracted PLFAs expressed as μg g^{-1} dry weight. Relative abundances of bacteria were determined by the abundance of specific biomarkers commonly used for this group. The sum of PLFAs considered to be predominantly of bacterial origin was further classified as Gram-positive bacterial (G^+) PLFAs (i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0) and Gram-negative bacterial (G^-) PLFAs (16:1ω7c, cy17:0, 17:1ω8, 18:1ω7c and cy19:0) (Frostegård and Bååth, 1996).

We determined the basal respiration of the microbial communities. The samples (2 g fresh weight) were placed in respiration vials, sealed and incubated at room temperature. Samples were incubated for 19 h. The amount of CO_2 produced was then determined by gas chromatography.

Bacterial growth was estimated by the leucine incorporation technique (Bååth, 1994), as modified by Bååth et al. (2001). Two grams of soil and 20 mL of distilled water were placed in 50-mL centrifuge tubes, shaken for 3 min on a vortex at high speed and then centrifuged at 1000 × g for 10 min, then 1.5-mL of each supernatant (bacterial suspension) were placed in microcentrifuge

tubes and incubated 2 h at 20 °C after addition of L-[4,5-^{3}H]-leucine (171 Ci mmolL^{-1}, 1.0 mCi mL^{-1}, Amersham) and non-radioactive leucine, to give a final concentration of 270 nM leucine. The incubation was stopped and the macromolecules were precipitated by adding 75 μL cold 100% trichloroacetic acid (TCA). Washing and preparation for scintillation counting was according to Bååth et al. (2001).

The fungal growth rate was assessed by the acetate in ergosterol method (Newell and Fallon, 1991) adapted for soil (Bååth, 2001). Briefly, 1 g of soil was transferred to test tubes to which 0.025 mL 1,2-[^{14}C]acetic acid (sodium salt, 2.04 GBq $mmol^{-1}$, 7.4 MBq mL^{-1}, Amersham), 0.475 mL 1 mM unlabelled acetate (pH = 6) and 1.5 mL distilled water were added, resulting in a final concentration of 0.22 mM acetate. The resulting soil slurry was incubated at room temperature (22 °C) for 8 h, after which 1 mL 5% formalin was added to terminate growth. Ergosterol was then extracted, separated and quantified by HPLC and a UV detector (282 nm), according to Rousk and Bååth (2007). The ergosterol peak was noted and the amount of incorporated radioactivity was determined in a liquid scintillation counter. The amount of ergosterol was used to estimate the fungal biomass-C.

2.4. Statistical analysis

The effect of plant genotype (*su1* and *sh2*), genetic background (I453x101t and P39xC23), type (inorganic, manure and vermicompost) and dose of fertilizer (standard and high) on nutrient contents and microbial communities of maize plants rhizosphere were analyzed by fitting mixed effects models in which the four above-mentioned factors were introduced as fixed effects and the effect of gene nested into block as random to account for any possible spatial effect. The analyses were performed with the lme function, from the nlme package. All main factors and interactions were included in the initial model and final models were obtained by backward elimination (Crawley, 2007). Comparisons between nearly isogenic hybrids (same genetic background) but with different endosperm mutations, and between hybrids with the same mutation but different genetic background enabled us to quantify the extent of effects on microbial communities due to changes in one gene, and those in the complete genotype, respectively. In order to analyze the underlying effect of the experimental factors on the rhizosphere microbial community, data from the PLFA analysis were subjected to discriminant analysis with the discriminant function from library ade4. The resulting scores from the first and second root of discriminant analysis were then tested to compare changes in the PLFA pattern by use of the above-mentioned mixed models. Data were analyzed using the R environment (R Development Core Team, 2007).

3. Results

The discriminant analysis of the 31 identified PLFAs showed that differences in one plant gene strongly shaped the structure of microbial communities of maize rhizosphere (Fig. 1). Thus, microbial communities from *su1* and *sh2* plants were clearly separated, except when plots received inorganic fertilization (Fig. 1; interaction fertilizer × gen, root 1, $F_{2,23} = 85.05$, $P < 0.001$ and root 2, $F_{2,23} = 9.06$, $P = 0.001$). These differences also depended on the dose of fertilizer (interaction fertilizer × dose × gen, root 1, $F_{2,23} = 16.72$, $P < 0.001$ and root 2, $F_{2,23} = 22.89$, $P < 0.001$), since in rhizosphere of *sh2* plants, high doses of fertilizer had a different effect on the microbial communities of inorganic and vermicompost-fertilized plots than on manure-fertilized plots. In the rhizosphere of *su1* plants, high doses of fertilizer modified microbial communities in different ways in the plots treated with inorganic fertilizer, manure and vermicompost.

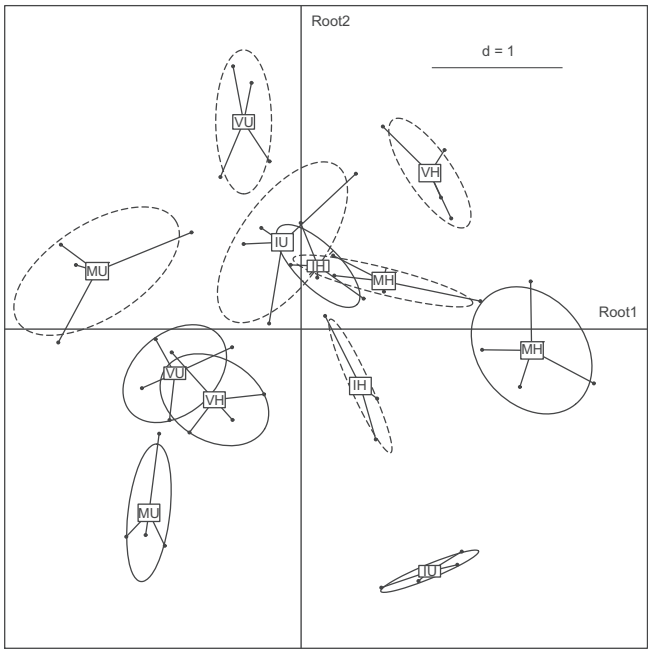

Fig. 1. Discriminant function analysis of 31 PLFAs identified from corn rhizosphere soil, as affected by plant genotype (*su1* (U) and *sh2* (H) genes), type of fertilizer (inorganic (I), manure (M) and vermicompost (V)) and dose of fertilizer (standard as solid line and high as dotted line), which are labelled inside their 95% inertia ellipses. The four points per group represent two replicates of each genetic background (I453x101t and P39xC23). Root 1 accounted for 31% and Root 2 for 29% of the total variance.

The living microbial biomass (total PLFAs content) only responded to dose of fertilizer, increasing significantly from standard (19.4 ± 1.18 μg g^{-1} dw) to high dose (23.1 ± 1.29 μg g^{-1} dw; $F_{1,39} = 5.50$, $P = 0.024$) as well as Gram-positive bacterial ($F_{1,39} = 6.14$, $P = 0.017$; 16.5 ± 1.06 and 19.9 ± 0.98 μg g^{-1} dw, standard and high doses respectively) and Gram-negative bacterial biomass ($F_{1,39} = 5.33$, $P = 0.026$; 14.6 ± 1.02 and 17.6 ± 0.84 μg g^{-1} dw, standard and high doses respectively). In addition, the biomass of Gram-positive bacteria was lower in rhizosphere of *su1* plants in the plots treated with inorganic fertilizer than in those treated with organic fertilizers, in contrast to the rhizosphere of *sh2* plants (interaction genotype × type of fertilizer, $F_{2,30} = 3.96$, $P = 0.029$; Fig. 2).

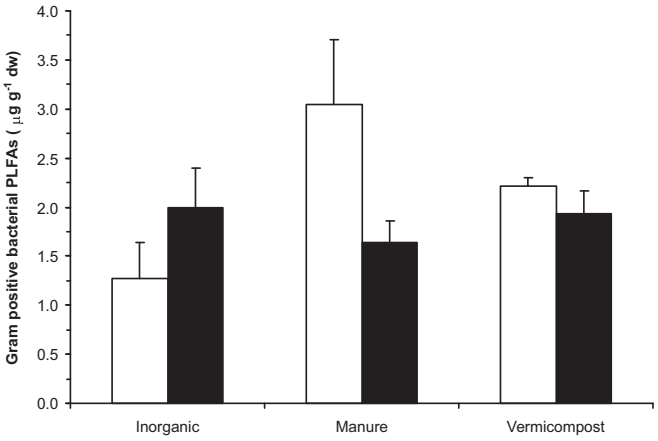

Fig. 2. Changes in Gram-positive bacterial PLFAs of maize rhizosphere as a function of type of fertilizer (inorganic, manure and vermicompost) and plant genotype (*su1* gen as white bars and *sh2* gen as black bars).

The fungal biomass-C did not vary between plant genotypes (1.51 ± 0.14 and 1.56 ± 0.12 μg g^{-1} dw for *sh2* and *su1* plants respectively) with the standard dose of fertilization, whereas it peaked in rhizosphere of *su1* plants with the high dose of fertilizer (2.35 ± 0.35 μg g^{-1} dw), with lower values in the rhizosphere of *sh2* plants (1.34 ± 0.10 μg g^{-1} dw) (interaction genotype × rate of fertilization; $F_{1,23} = 7.32$, $P = 0.012$). Fungal biomass-C peaked with the high dose of fertilizer in all genotype × genetic background combinations, except for *sh2* × I453x101t (interaction genotype × genetic background × dose of fertilizer, $F_{1,23} = 6.71$. $P = 0.016$; Fig. 3a). In addition, fungal biomass-C was lower with the standard dose of fertilizer, a trend found for the two-plant genotypes in soils treated with inorganic fertilizer and for *su1* plants in soils fertilized with manure, whereas in vermicompost-fertilized plots and manure-fertilized plots there were no differences due to dose of fertilizer (interaction genotype × fertilizer × dose of fertilizer, $F_{2,23} = 3.96$. $P = 0.033$; Fig. 3b). The trend was similar for genetic background, with lower values of fungal biomass-C with all the three fertilizers, although there was an increase from high to standard dose of fertilizer (vermicompost with I453x101t) and no effects at all (inorganic with P39xC23) (interaction genetic background × fertilizer × dose of fertilizer, $F_{2,23} = 4.86$. $P = 0.017$; Fig. 3c).

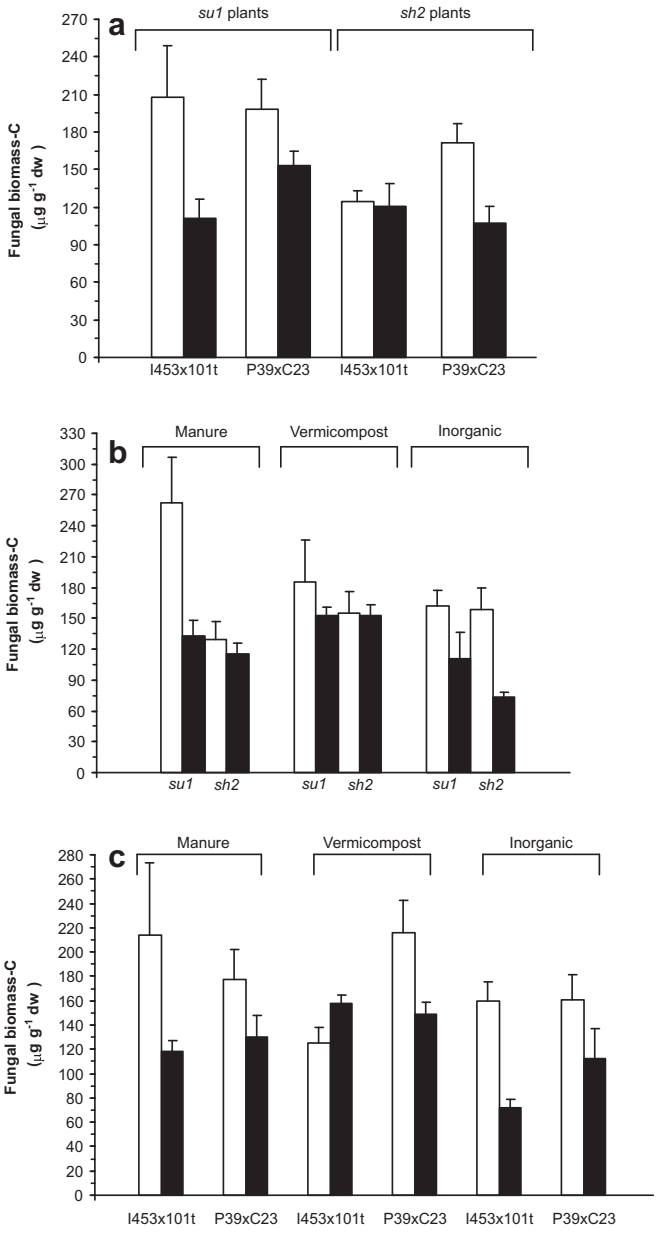

Fig. 3. Changes in fungal biomass-C estimated from ergosterol content as a function of a) plant genotype (*su1* and *sh2* genes), genetic background (I453x101t and P39xC23) and dose of fertilizer (high as white bars, and standard as black bars), b) plant genotype (*su1* and *sh2* genes), type of fertilizer (manure, vermicompost and inorganic) and dose of fertilizer (high as white bars, and standard as black bars), and c) plant genetic background (I453x101t and P39xC23), type of fertilizer (manure, vermicompost and inorganic) and dose of fertilizer (high as white bars and standard as black bars). Mean ± S.E.M.

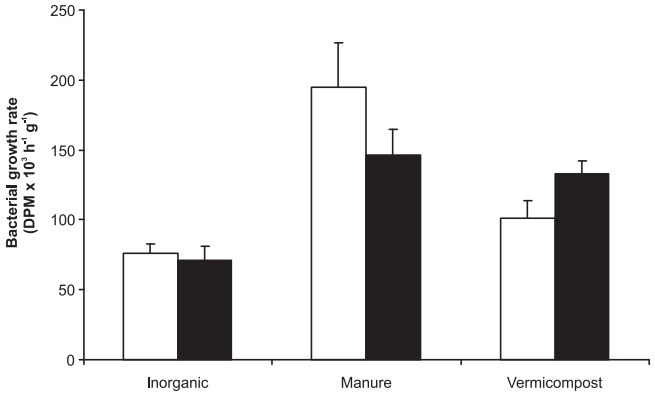

Fig. 4. Changes in bacterial growth rate (leucine incorporation) of maize rhizosphere as a function of plant genotype (*su1* gen as white bars and *sh2* gen as black bars) and type of fertilizer (inorganic, manure and vermicompost). Mean ± S.E.M.

Microbial activity, measured as basal respiration, did not depend on plant genotype or fertilizer type, but responded significantly to the dose of fertilizer ($F_{1,39} = 8.77$, $P = 0.005$), with higher values in response to the high dose (4.57 ± 0.35 μg CO_2 g^{-1} dw h^{-1}) than in response to the standard dose (3.05 ± 0.14 μg CO_2 g^{-1} dw h^{-1}).

Bacterial growth in the rhizosphere of *su1* plants peaked in plots fertilized with manure, whereas in *sh2* plants, it peaked in plots fertilized with vermicompost (Fig. 4; interaction genotype × fertilizer, $F_{2,30} = 4.31$, $P = 0.02$). Moreover, the bacterial growth rates depended on the type of fertilizer, with rates increasing from inorganic (73 ± 5 × 10^3 DPM h^{-1} g^{-1}) and, vermicompost (117 ± 8 × 10^3 DPM h^{-1} g^{-1}) to manure amendments (170 ± 18 × 10^3 DPM h^{-1} g^{-1}) ($F_{2,30} = 23.26$, $P < 0.0001$). In addition, the bacterial growth rate responded positively to the rate of application of organic fertilizers, with increasing values from standard (93 ± 10 × 10^3 DPM h^{-1} g^{-1}) to high dose (147 ± 13 × 10^3 DPM h^{-1} g^{-1}) ($F_{1,30} = 22.26$, $P = 0.0001$). The growth rate of fungi was significantly affected only by the dose of fertilizer ($F_{1,30} = 23.59$, $P < 0.0001$), and was lower in the plot to which the standard dose was applied (70 ± 3 DPM h^{-1} g^{-1}) than when the high dose was added (101 ± 6 DPM h^{-1} g^{-1}).

4. Discussion

The presence of two genes responsible for encoding the amount and type of sugar in the endosperm, and likely the amount and type of sugar flowing to the rhizosphere, was shown to have strong and clear effects on rhizosphere microbial community structure, physiology, and activity. Several previous studies have shown changes in rhizosphere communities due to changes in maize genotype, but our results are unique in that we have shown that differences in plant genotype related to C plant metabolism may shape the rhizosphere microbial communities. Further, fertilization was also shown to strongly influence rhizosphere microbial communities, raising levels of microbial biomass of bacteria and fungi. More

important, plant genotype modulated the effects of fertilization resulting in different microbial communities.

4.1. Genotype effects on rhizosphere microbial communities

We found that *su1* and *sh2* plants promoted different microbial communities in the maize rhizosphere. In this way, Brusetti et al. (2004) found that rhizosphere microbial communities in transgenic Bt 176 maize plants differed from those in the non-transgenic counterpart. However, Chiarini et al. (1998) did not find any differences in rhizosphere microbial communities in several varieties of maize, and found that soil type was the main factor affecting rhizosphere microbial communities. There were no plant genotype effect on the abundance of microbial main groups (bacteria, Gram-positive and negative bacteria and fungi), which concords with those reported in previous studies (Prischmann et al., 2008; Roesch et al., 2006; Toljander et al., 2008). This data indicate that plants modified the composition and not the abundance of microorganisms suggesting that differences in composition of root exudates may be responsible of these changes.

4.2. Fertilization effects on rhizosphere microbial communities

As plant genotype, the use of different fertilizers and doses modified rhizosphere microbial communities, as showed Toljander et al., (2008) in a comparison of several organic and inorganic fertilizers. Although we added the same amount of N, P and K to soil, the three fertilizers (inorganic, manure and vermicompost) differed strongly in their chemical composition. Inorganic fertilization only supplied N, P and K, whereas organic fertilizers also supplied different amounts of C and macro and micronutrients (Edwards, 2004), which could have selected for microbial communities with different nutritional requirements (Tate, 2000). Furthermore, these nutritional differences would be more important if the plants were sampled when production of root exudates was lower or had ceased (Neumann and Römheld, 2007). In addition, microbial communities in vermicompost are metabolically more diverse than those in manure (Aira et al., 2007), and may be incorporated, at least in the short term, to soils (Gómez et al., 2006). These changes in microbial metabolic diversity are associated with modifications in microbial community structure (Andersen et al., 2010; Kohler et al., 2005). This is important since the soil microbial pool will determine the rhizosphere microbial community (Berg and Smalla, 2009; Girvan et al., 2003). Thus, the application of organic fertilizer resulted in different microbial communities from those that develop after the application of inorganic fertilizer (Toljander et al., 2008), although some of these effects have only been found in long-term experiments (Esperschütz et al., 2007; Toljander et al., 2008; but see Chu et al., 2007). It is important to note that in the present study, the effect of the addition of organic fertilizer occurred despite the low dose of organic fertilizer used (25% of total fertilization), and despite the short duration of the experiment (four months).

Fungal growth only responded to the dose of fertilizer whereas bacterial growth also increased with organic fertilization. This may be explained by the different C:N ratios in manure and vermicompost (15.8 and 11.1 respectively), so that the addition of only inorganic fertilizer would result in even lower values (initial soil C:N ratio was 8.9), which may have reduced fungal growth (Tate, 2000). The results are not consistent with the fact that organic fertilizers with higher C:N ratios enhance fungal growth (Thiet et al., 2006). However, it has recently been shown that the addition of N may increase fungal growth but decrease bacterial growth, although the growth rates returned to initial values after 60 days (Rousk and Bååth, 2007).

4.3. Interaction genotype × fertilization effects on rhizosphere microbial communities

Despite of marked effects that plant genotype and fertilization exerted separately, in the present study, the larger differences in the microbial communities in the maize plant rhizosphere were mainly due to changes in fungal and Gram-positive bacteria biomass. We sampled the rhizosphere when the corncobs had matured (i.e. when release of root exudates has decreased or ceased and microorganisms must therefore obtain their nutrients from soil) (Brimecombe et al., 2007). In this case, if plant genotype did not affect microbial structure, we would expect that microbial communities would be closely grouped depending on the type of fertilizer, because of differences in the nutrient contents (other than N, P and K) of the three fertilizers. However, the same fertilization treatment produced different microbial communities that clearly depended on plant genotype, probably due to adaptation of microbial communities to root exudates prior to plant senescence, which would have determined the structure of the rhizosphere microbial community.

Gram-positive bacteria and bacterial growth varied with plant genotype and type of fertilizer. This may be due to the faster response of saprotrophic bacteria than that of the slower saprotrophic fungi, which can proliferate in the short term when there is a readily available source of energy (Cardon and Gage, 2006), as in the case of the organic treatments. As microorganisms are usually limited by carbon in soil (Demoling et al., 2007), this may obviously be explained by the additional amounts of carbon supplied by the organic fertilizers, and even indirectly through the effects of fertilizers that would modify the plant root exudates. Lazcano et al. (submitted for publication) found that plant biomass was higher in *sh2* than in *su1* plants, with no differences among fertilizers in *sh2* plants and with lower plant biomass in vermicompost than manure-fertilized *su1* plants (interaction plant genotype × fertilizer, $P = 0.03$). However, microbial parameters like biomass and growth rates showed the opposite trend, indicating that larger plants did not promote bacterial and fungal biomass and growth. This suggests that the effects of plant genotype will be due to differences in composition and/or quality rather than to the amount of root exudates released by plants.

5. Conclusions

We found that the variation in two-plant genes (*su1* or *sh2*) responsible for plant C allocation strategy greatly modified the structure and activity of the microbial community of maize rhizosphere. Further, these changes in microbial community structure occurred without modifications in the biomass of main microbial groups which indicate that plants effectively supported their rhizosphere microorganisms and suggest that differences in root exudates may be responsible of these effects. Although microorganisms clearly responded to dose of fertilization, the three fertilizers (inorganic, manure and vermicompost) also contributed to differentiate rhizosphere microbial communities as well as the abundances of specific microbial groups, mainly due to their different nutrient contents and microbial load. Moreover, larger plants did not promoted higher biomass or microbial growth rates suggesting complex interactions between plants and fertilizers, probably as a result of the different performance of plant genotypes within fertilizer treatments, i.e. differences in the quality and/or composition of root exudates.

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