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Short communication

Carnivory does not change the rhizosphere bacterial community of the plant *Drosera intermedia*

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ABSTRACT

Carnivorous plants are also green plants, and some are even able to grow and reproduce in the absence of prey, which implies that they should be able to capture nutrients from their roots. We studied the rhizosphere bacterial community of the carnivorous plant *Drosera intermedia* and examined whether it is influenced by the plant nutrient source, i.e., soil or preys. Notably, we did not observe any differences in the bacterial communities of plants after ten weeks feeding on prey or soil. Further, rhizosphere nutrient pools (mineral N and P and organic N and C) were not affected by plant feeding regime. We found that *D. intermedia* has a well-defined rhizosphere bacterial community, mainly composed by OTUs from phyla *Proteobacteria* (40%), *Acidobacteria* (27%), *Actinobacteria* (9%) and *Firmicutes* (5%). This community is richer (alpha diversity) than the bulk soil bacterial community at the taxonomic, although there was no differences in phylogenetic diversity. We also found that these communities differed when considering qualitative (presence–absence of OTUs) and quantitative (abundance of OTUs) measurements at taxonomic and phylogenetic levels. These results, together with the lack of effect due to feeding regime, suggest that rhizosphere bacterial community of *D. intermedia* is metabolically diverse to cope with changes in plant physiology condition.

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1. Introduction

Carnivory in plants is assumed to have evolved to bypass living in poor nutrient soils (Ellison and Gotelli, 2001), but carnivorous plants are also green plants (C3 metabolic pathway; Lüttge, 1983). Interestingly, both ways of nutrient acquisition (from soil and preys) are linked in these plants, and thus predation may enhance the absorption of soil nutrients (Adamec, 1997, 2002). Carnivorous plants can also reallocate nutrients from senescent leaves or storage organs to cope with lack of prey and/or low soil nutrient availability (Schulze and Schulze, 1990; Adamec, 2002). Therefore, carnivorous plants show a high degree of plasticity in their feeding habits, even during the same season.

The rhizosphere is the biologically active zone of soil where plant roots and microorganisms interact. Since a variable amount of photosynthesized C is exudated (10–44%) (Marschner, 1995; Bais et al., 2006) changes in the availability and quality of root exudates should modify the composition of rhizosphere microbial communities (Dennis et al., 2010). In addition, the plant nutrient requirements also modify rhizosphere nutrient pools altering rhizosphere microbial communities (Bardgett and Wardle, 2010).

The carnivorous plant *D. intermedia* has specific physiological characteristics that make it suitable for studying how changes in feeding strategies affect the rhizosphere bacterial community: (i) a well-defined root system that can extend up to 6 cm (Crowder et al., 1990), (ii) ability to absorb nutrients from soil, which may be partially enhanced after prey capture, (iii) poor nutrient (N and P) re-utilization from plant living tissue (reviewed by Adamec, 1997) and (iv) the ability of grow and reproduce without catching preys (Wilson, 1985; Crowder et al., 1990). Therefore, plants deprived of preys should obtain their nutrients from soil, and consequently soil nutrient absorption should be higher relative to plants that catch preys. Such changes in nutrient acquisition strategies, and therefore in soil nutrient pools, should be reflected in changes in the rhizosphere bacterial community structure.

2. Material and methods

2.1. Sample collection and experimental design

We collected 20 specimens of *D. intermedia* Hayne (1800) from a peatland (42°19′40.51″N, 8°21′9.81″W, Spain). Plants were potted

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with their soil and kept in a thermostatically controlled glasshouse; we also collected 10 soil samples without plants to analyze them as bulk soil. Prior to the start of the experiment, all preys were removed from all plants. Ten plants were assigned to the soil-derived treatment, in which plants did not receive any prey, and the other ten plants were assigned to the prey-derived treatment, in which each leaf on the plants was fed with one *Drosophila melanogaster* specimen obtained from laboratory cultures. One fly was also placed on each new leaf that appeared on the prey-derived plants. We sampled bulk and rhizosphere soil after ten weeks. Soil samples were separated for nutrient content analysis and DNA extraction (frozen at −80 °C).

2.2. Soil nutrient content analysis

The soil samples were dried at 105 °C for 24 h for determination of the moisture content. Inorganic N ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$), dissolved organic N and C were determined in 0.5 M K_2SO_4 extracts (1:5 weight/volume) by colorimetric methods. Phosphate was extracted by digesting soil samples with acetic acid, filtered and determined by colorimetric methods.

2.3. Soil DNA extraction and bar-coded pyrosequencing

Soil DNA (0.25 g) from rhizosphere and bulk soil samples was extracted with the PowerSoil DNA Isolation kit (MoBio Laboratories Inc., Carlsbad, California) according to the manufacturer's protocol. Only 9, 6 and 8 samples were successfully amplified from soil derived plants, prey-derived plants and bulk soil, respectively. We amplified a fragment of 16S rRNA covering V2 (forward 5'-AGYGGCGIACGGGTGAGTAA) and V3 (reverse 5'-ATTACCGCGGCTGCTGG) regions by using primers and a touch-down PCR protocol described by Sundquist et al. (2007). Following successful amplification samples were submitted to the sequencing center at Brigham Young University where they were cleaned of primer dimer using AMPure beads and pooled in equal amounts according to the total quantity of DNA as estimated with Quant-iT PicoGreen and sequenced using a Roche 454 sequencer.

2.4. Processing of pyrosequencing data

Data from sff files were processed with mothur and the default settings described by Schloss, et al. (2011). Sequence reads were first trimmed to remove barcode and primer sequences and yield sequences with a minimum of 200 bp that were then aligned to the bacterial-subset SILVA alignment. Chimeras were checked and removed. Sequences were classified with the naïve Bayesian classifier against a RDP reference file version 10 provided by mothur (http://www.mothur.org/wiki/RDP_reference_files). Operational taxonomic units (OTUs) at the 0.03 level were obtained by constructing a distance matrix and then clustering the resulting sequences into OTUs. Sequence data (raw sff files) have been submitted to the GenBank SRA database under accession number SRP036172.

2.5. Statistical analysis

2.5.1. Soil nutrient analysis

All chemical variables but one (phosphates were log-transformed) met normality and homoscedasticity assumptions, and means of rhizosphere of soil-derived and prey-derived plants were separated with *t*-tests.

2.5.2. Sequence analysis

In order to remove the effect of sample size on community composition, we rarefied samples to 159 sequences to study prey-derived (4 samples) and soil-derived rhizosphere bacterial communities (9 samples), and to 300 sequences to study bulk soil (8 samples) and rhizosphere bacterial communities (7 samples). We inferred an approximately-maximum-likelihood phylogenetic tree by using FastTree 2.1 (Price et al., 2010). The taxonomic alpha diversity was estimated as richness, with the observed number of OTUS (sobs) and the non-parametric estimator Chao1 to estimate the true species diversity. Diversity was estimated by the Shannon index. Phylogenetic diversity was calculated as Faith's phylogenetic diversity (Faith, 1992). The four measurements of alpha diversity met normality and homoscedasticity assumptions for samples of rhizosphere (soil-derived and prey-derived plants) and bulk soil. We used *t*-tests to determine any significant difference in alpha diversity of bacterial communities from (i) rhizosphere of soil-derived and prey-derived plants and (ii) rhizosphere and bulk soil samples.

Taxonomic beta diversity was estimated as differences in bacterial taxonomic community composition at OTU level between samples of rhizosphere (soil-derived vs. prey-derived) and between samples of rhizosphere and bulk soil. This was done by principal coordinate analysis (PCoA) using distance matrix that taking into account the abundance of OTUs (Bray–Curtis) or not (Jaccard). Phylogenetic beta-diversity was also calculated by PCoA with weighted (considering abundance of OTUs) and unweighted UniFrac distances, which were obtained as averages after sampling the phylogenetic tree 1000 times. Taxonomic and phylogenetic community composition was compared between rhizosphere samples (soil-derived vs. prey-derived) and between rhizosphere and bulk soil samples with ANOSIM tests. Data are presented as mean ± SE. Full detailed methods are provided in the Supplementary data.

3. Results

After processing sff files, we obtained 5977 sequences, which resulted in 543 OTUs defined at 97% similarity, 259 for prey-derived and 424 for soil-derived plants. However, sampling depth was suboptimal as non-saturated rarefaction curves showed (Supp. Fig1a). There were no differences in alpha-diversity (taxonomic and phylogenetic) between rhizosphere bacterial

Table 1
Comparison of alpha diversity measurements of bacterial communities from (i) rhizosphere of *Drosera intermedia* plants that were obtained nutrient from soil (sol-derived) or preys (prey-derived) and (ii) rhizosphere of *D. intermedia* plants and bulk soil. Taxonomic alpha diversity was measured as OTU richness (number of OTUs found), estimated richness (Chao1) and diversity (Shannon index), phylogenetic diversity was measured as Faith index. Mean ± SE.

	Prey-derived rhizosphere	Soil-derived rhizosphere	Rhizosphere soil	Bulk soil
OTU richness (sobs)	87 ± 1	91 ± 4	135 ± 2	123 ± 3
Chao1 richness	245 ± 23	238 ± 11	323 ± 9	277 ± 6
Shannon index	4.09 ± 0.02	4.19 ± 0.05	4.39 ± 0.04	4.22 ± 0.06
Faith's index	1.46 ± 0.73	1.46 ± 0.47	17.38 ± 0.72	15.64 ± 0.75

communities of soil-derived and prey-derived plants ($P > 0.05$ for all measurements, Table 1). There were no differences in either taxonomic or phylogenetic beta-diversity, with bacterial communities in samples from soil-derived and prey-derived plants clearly overlapping in all PCoA analysis (Supp. Fig. 2). There were no differences in mineral nitrogen ($P = 0.2$ and $P = 0.8$ for ammonium and nitrate), dissolved organic nitrogen ($P = 0.18$) and carbon ($P = 0.3$) and phosphate contents ($P = 0.9$) between rhizosphere from prey-derived and soil derived plants.

After processing raw files, we obtained 13,443 sequences, which yielded 834 OTUs defined at 97% similarity, 310 for bulk and 351 for rhizosphere soil samples. However, sampling depth was not good enough as non-saturated rarefaction curves confirm (Supp. Fig 1b). Bacterial populations in the *D. intermedia* rhizosphere, grouped into 13 phyla, differed in composition from those of bulk soil (Fig. 1). Thus, at phylum level, the main differences in shared phyla were due to decreases in the abundance of OTUs from phyla Firmicutes (2.56-fold decrease in abundance from bulk to rhizosphere), Verrucomicrobia (1.75-fold) and Acidobacteria (1.24-fold) and increases in Actinobacteria (1.10-fold increase in abundance from rhizosphere to bulk), Bacteroidetes (1.94-fold), Chloroflexi (1.20-fold), *Proteobacteria* (1.56-fold), and candidate phyla TM7 (4.69-fold) and WS3 (1.14-fold). Moreover, several phyla only appeared in the rhizosphere (*Planctomycetes, Nitrospira* and Chlorobi), whereas *spirochaetes* was only present in bulk soil samples. Rhizosphere bacterial communities of *D. intermedia* plants were more diverse (Shannon index; $P = 0.046$) and were also richer (Sobs; $P = 0.01$); the same was true for estimated richness (Chao1, $P < 0.001$). However, phylogenetic diversity only tended to be higher in the rhizosphere than in bulk soil samples ($P = 0.12$)(Table 1). Microbial communities of bulk soil and *D. intermedia* rhizosphere differed at taxonomic (Fig. 2a; ANOSIM, $R = 0.89$, $P < 0.001$) and phylogenetic beta-diversity levels (Fig. 2b; ANOSIM, $R = 0.67$, $P < 0.0001$) without considering abundance of OTUs; the same was true when abundances were considered at both taxonomic and phylogenetic levels (Supp. Fig. 3). In both cases, dissimilarities within samples were higher in rhizosphere samples than in bulk soil samples (Supp. Figs. 3 and 4).

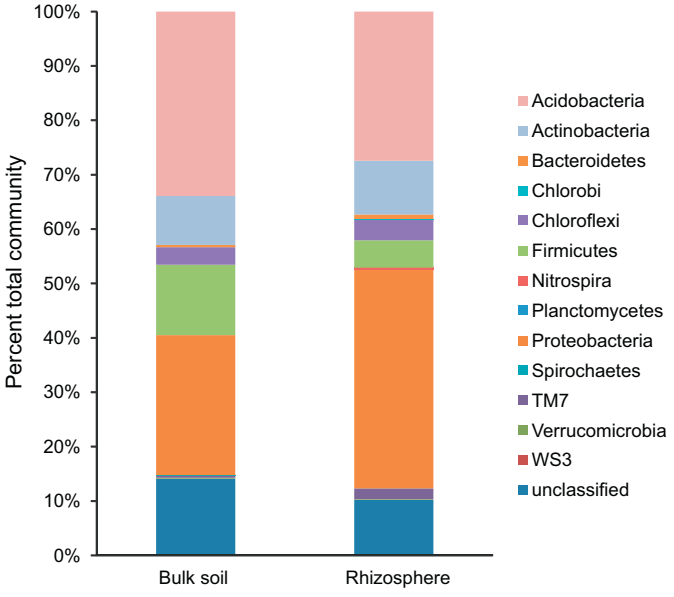

Fig. 1. Bacterial community composition of bulk soil and rhizosphere of *Drosera intermedia* at phylum level.

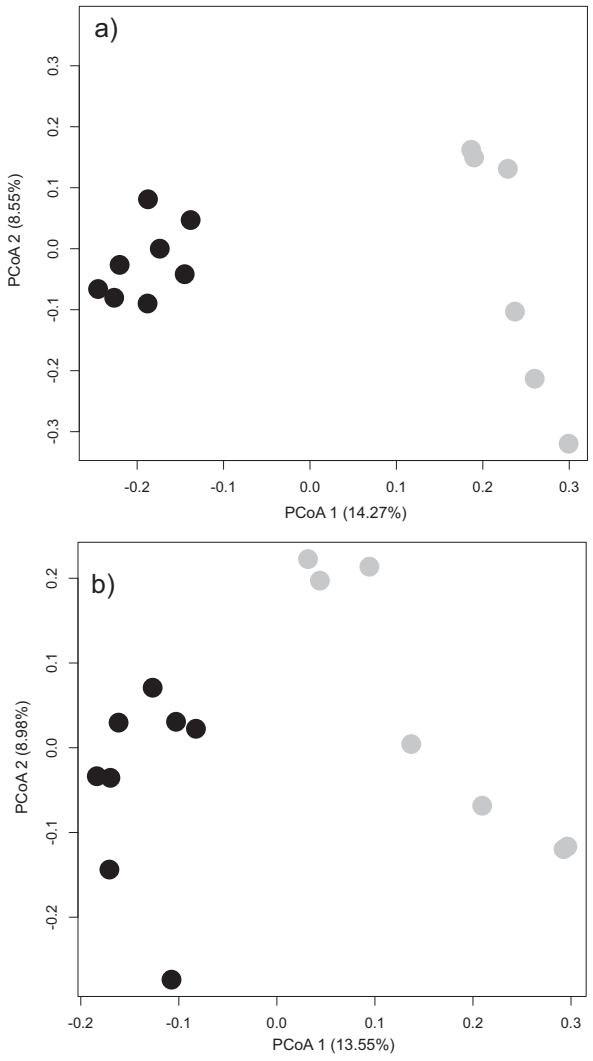

Fig. 2. Principal coordinate analysis of (a) taxonomic (Jaccard), and (b) phylogenetic (unweighted UniFrac) beta-diversity of bacterial communities in the bulk soil ($n = 8$, black symbols) and rhizosphere of *Drosera intermedia* plants ($n = 7$, grey symbols).

4. Discussion

We found that the carnivorous plant *D. intermedia* possess a well-defined rhizosphere bacterial community, which is different from the bacterial community of the bulk peat soil. More importantly, we also found that the composition of this bacterial community was not influenced by a change in nutrient source during ten weeks. The lack of prey should have switched plant nutrient acquisition from prey to soil, and increase photosynthesis rates. This should enhance soil nutrient absorption as well as root exudation by plant roots (Adamec, 2002). However, there were no differences in the rhizosphere nutrient pools analyzed. Our results suggest that rhizosphere bacterial communities are relatively independent of *D. intermedia* plants. In this way, it has been shown that in rhizosphere of *Drosera villosa* there is a high diversity of diazotrophic bacteria (Albino et al., 2006). If this were true also for *D. intermedia*, rhizosphere bacteria could gain benefit accessing to fixed N_2 by diazotrophs. However, we did not find any increase in OTUs with known N_2 fixing capabilities (data not shown). In fact, most of possible diazotrophs showed higher abundances in bulk soil than in rhizosphere soil. Since *D. intermedia* roots show different degrees of colonization by arbuscular mycorrhizal fungi (Fuchs and Haselwandter, 2004; Weishampel and Bedford, 2006)

we should consider the possibility that mycorrhizas have played a role, either by supporting soil-derived plants or by acting as a sink for fixed C and thus being favored over rhizosphere bacteria, as proposed by Drigo et al. (2010).

Peatland ecosystems are known to harbor metabolically diverse microbial communities (Andersen et al., 2013). We found that *Proteobacteria* and *Acidobacteria* were the most abundant bacterial phyla in our samples, both in the bulk (216 and 33%) and rhizosphere soil (40 and 27%). These two bacterial phyla usually dominate bacterial communities of peatlands, with abundances ranging between 28–55% and 17–63% (for *Proteobacteria* and *Acidobacteria*, respectively; Pankratov et al., 2011; Lin et al., 2012; Serkebaeva et al., 2013). We found that the less well represented phyla such as *Planctomycetes* (0.04%), *Chlorobi* (0.04%) and *Bacteroidetes* (0.6%) were less abundant, than in previous studies, where their abundances were higher (*Planctomycetes* (2–5%), *Chlorobi* (2%) and *Bacteroidetes* (1%); same references as above). This may be due to the low sampling representativeness achieved (sequence sampling), as in previous pyrosequencing based studies of bacterial communities of peatlands between 23 and 53 bacterial phyla were found (Lin et al., 2012; Serkebaeva et al., 2013).

Interestingly, the alpha diversity was higher in the rhizosphere bacterial communities than in the bulk soil at taxonomical, but not at phylogenetic level. Phylogenetic diversity has been shown to be a better predictor of ecosystem functioning than species richness, in several studies (reviewed in Cadotte et al., 2008). Therefore, the lack of any differences in phylogenetic diversity suggests that bacterial communities are subjected to strong selective pressures in peatland ecosystems. Furthermore, the rhizosphere bacterial community of *D. intermedia* differs (beta diversity) at both taxonomical and phylogenetic levels from those of bulk soil. We observed this at both quantitative and qualitative levels. Quantitative changes imply differences in the abundance of OTUs between the two environments; such changes may be related to temporal factors (Lozupone et al., 2007), such as physiological plant state and nutrient availability, all of which are limiting factors for the abundance of OTUs. On the other hand, qualitative changes imply that specific OTUs thrive exclusively in the rhizosphere or in the bulk soil. Qualitative differences may be due to factors related to microbial growth or different founder bacterial populations (Lozupone et al., 2007), which are involved in the establishment of plants in bulk soil. Moreover, these differences still appear when phylogenetic information is incorporated, indicating that the bacterial communities of both environments are phylogenetically distant, reflecting specific adaptations necessary to succeed either in bulk or rhizosphere soil (Lozupone et al., 2007). The present findings show that relationships between plants and their rhizosphere bacterial communities are far from being understood, especially for species living in stressful environments.

We described, for the first time, the carnivorous plant *D. intermedia* rhizosphere bacterial community. Interestingly, obtaining nutrients from soil did not modify the bacterial communities, which suggest that the rhizosphere bacterial community of *D. intermedia* is metabolically diverse to cope with changes in plant physiology condition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2015.03.001.

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