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▶ **To cite this version:**

Wafa Achouak, Danis Abrouk, Julien Guyonnet, mohamed Barakat, Philippe Ortet, et al.. Plant hosts control microbial denitrification activity. FEMS Microbiology Ecology, 2019, 95 (3), pp.fiz021. 10.1093/femsec/fiz021 . cea-02096084

HAL Id: cea-02096084
https://cea.hal.science/cea-02096084

Submitted on 11 Apr 2019

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Plant hosts control microbial denitrification activity

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ABSTRACT

In the rhizosphere, complex and dynamic interactions occur between plants and microbial networks that are primarily mediated by root exudation. Plants exude various metabolites that may influence the rhizosphere microbiota. However, few studies have sought to understand the role of root exudation in shaping the functional capacities of the microbiota. In this study, we aim to determine the impact of plants on the diversity of active microbiota and their ability to denitrify *via* root exudates. For that purpose, we grew four plant species, *Triticum aestivum*, *Brassica napus*, *Medicago truncatula* and *Arabidopsis thaliana,* separately in the same soil. We extracted RNA from the root-adhering soil and the root tissues, and we analysed the bacterial diversity by using 16S rRNA metabarcoding. We measured denitrification activity and denitrification gene expression (*nirK* and *nirS*) from each root-adhering soil sample and the root tissues using gas chromatography and quantitative PCR, respectively. We demonstrated that plant species shape denitrification activity and modulate the diversity of the active microbiota through root exudation. We observed a positive effect of *T. aestivum* and *A. thaliana* on denitrification activity and *nirK* gene expression on the root systems. Together, our results underscore the potential power of host plants in controlling microbial activities.

Keywords: Rhizosphere, Denitrification, Root, Root-adhering soil, Active microbiota, Root exudates, Nitrogen uptake.

INTRODUCTION

The ability to secrete a wide range of compounds into the rhizosphere is one of the most remarkable metabolic features of plant roots with approximately 5 to 21% of the total photosynthetically fixed carbon being transferred into the rhizosphere through root exudates (Whipps, 1990; Marschner, 1995; Nguyen, 2003). These compounds are metabolized by soil-borne microorganisms as carbon and energy sources providing the basis for the establishment of plant-microorganism interactions that benefit plant growth by increasing the availability of mineral nutrients, production of phytohormones, degradation of phytotoxic compounds and suppression of soil-borne pathogens (Bais *et al*., 2006; Philippot *et al*., 2013; Haichar *et al.,* 2014). This demonstrates the importance

of studying the functional properties of the soil microbiota and the manner in which plant species influence bacterial diversity and microbial activities.

Denitrification is one of the microbial activities that occurs in the plant rhizosphere. Denitrification is the microbial process of the nitrogen cycle that allows the return of fixed nitrogen (reduction of atmospheric N_2) to the atmosphere. The reduction of soluble NO_3^- or NO_2^- into gas (NO, N_2O or N_2) by denitrification is catalysed by metalloenzymes: nitrate reductases (Nar and Nap), nitrite reductases (NirK and NirS), nitric oxide reductases (cNor and qNor) and nitrous oxide reductase (Nos) (Zumft, 1997; Philippot, 2006). This stepwise reduction of nitrate is an alternative respiration pathway occurring in the case of oxygen depletion by phylogenetically diverse microorganisms in a wide range of ecosystems (Zumft, 1997). Most denitrifiers belong to various subclasses of bacteria, although the ability to denitrify has also been found in some archaea and fungi (Philippot, 2002a). In addition, co-occurrences of denitrification genes do not appear to be randomly distributed among taxonomic groups (Graf *et al.,* 2014).

Denitrification represents a significant loss of nitrogen in soils (25–90%) (van der Salm *et al*., 2007; Radersma & Smit, 2011), and this microbial process therefore affects the availability of nitrogen to plants. This process contributes to N_2O emission, a powerful greenhouse gas (300-fold more heat-trapping capacity than CO_2, per molecule) and the single most important ozone-depleting agent known (Ravishankara *et al*., 2009; Coskun *et al*., 2017).

The major factors regulating denitrification can be modulated in the rhizosphere: nitrate concentration (via absorption by plants) and oxygen partial pressure (via root respiration and the surrounding root moisture) are decreased, while the C availability (*via* rhizodeposition) is generally increased (Tiedje, 1988; Mounier *et al.,* 2004; Langarica-Fuentes *et al*., 1918). The impact of the rhizosphere on denitrifying activity has already been reported (Mahmood *et al.,* 1997; Mounier *et al.,* 2004; Patra *et al.,* 2006; Henry *et al.,* 2008). For example, Philippot *et al.* (2002b) observed that the presence of maize roots led to a change in the structure of the nitrate-reducing community. However, little is known about how denitrification is regulated in the rhizosphere. Determining the role of host plant on denitrification process modulation is of real importance in understanding the regulation of denitrification in the rhizosphere.

The aim of this study was to determine the impact of four plant species, wheat (*Triticum aestivum*), rape (*Brassica napus*), barrel clover (*Medicago truncatula*) and *Arabidopsis thaliana* (ecotype Colombia), cultivated in the same soil on i) the diversity of the bacterial community by metabarcoding the 16S rRNA gene genes, and

on ii) the activity of the denitrifying community in the root-adhering soil (RAS) and on the root system by evaluating the denitrification activity and *nirK* and *nirS* gene expression.

MATERIAL AND METHODS

Plant growth

A laboratory experiment was performed with winter wheat (*T. aestivum* L. cv. Taldor), rape (*B. napus* cv. Drakkar), *A. thaliana* (ecotype Colombia) and barrel clover (*M. truncatula*, ecotype A17) on a calcareous silty-clay soil collected from the upper 20 cm layer of the agricultural site located in the Aix en Provence region. The pH was 8.2 and it contained 5.7% sand, 46.7% silt, 47.6% clay, 1.0% $CaCO_3$, 1.8% organic C and 0.18% organic N. The soil was sieved (1-mm mesh size), air-dried, and the water holding capacity (WHC) measured according to Bouyoucos (1929) based on the pulling out of water from soil by suction or vacuum forces. The WHC was determined in triplicates and was about 7.82%. 150 g dry soil was placed into polypropylene cylindrical pots. Soil moisture was maintained at 75% of WHC by adding the necessary amount of water. Seeds were sterilized according to Achouak *et al.* (2004) and one seed was planted per pot. Sixteen pots of each plant were grown in a growth chamber. 4 pots (4 repetitions) per plant were dedicated to denitrification activity measurements (on the RAS and the root system), 3 pots (3 repetitions) per plant were dedicated to molecular analysis (16S rRNA diversity from the RAS and the root system) and 9 pots per plant were dedicated to isotope labelling experiment. Sixteen pots with soil but without plants (bulk soil treatment) were used: 4 pots (4 repetitions) were dedicated to denitrification activity measurements, 3 pots (3 repetitions) were dedicated to molecular analysis (16S rRNA diversity) and 9 pots were dedicated to isotope labelling experiment. Plants and bulk soil pots were incubated in a growth chamber with a day–night period about 12/12, respectively; light intensity was 400 mmol photon $m^{-2}.s^{-1}$ and maximum daily temperature ranged from 20 to 22 °C. Soil moisture was manually controlled.

Plant harvesting

After 5 weeks of plant growth, plants were collected as follows: the root system of 3 replicates of each plant were separated from its root-adhering soil (RAS) and 2 g of RAS from each plant were frozen in liquid N_2 immediately and stored at -80 °C for molecular analysis. The root systems were washed with water to remove adhering soil particles, frozen in liquid N_2 and stored at -80 °C. The bulk soil microcosms (4 replicates) were also collected for

106 microbial activities measurements and 2 g were frozen in liquid N_2 immediately and stored at -80 °C for molecular
107 analysis.

108 The entire plant from 4 replicates for each plant was separated from the RAS and both, the whole plant and the
109 RAS of the planted microcosm and soil of the unplanted microcosm were used to measure denitrification activity.

110 The remaining 9 replicates for each plant and the bulk soil treatment were used for isotope labelling experiments.

111

112 ***Measurements of microbial denitrification activities***

113 ***Denitrification Enzyme Assay without carbon addition***

114 We measured the denitrification activity of microbial communities inhabiting the root system in each plant
115 rhizosphere in 4 replicates according to Guyonnet *et al.* (2017). The entire plant with the root system, rinsed with
116 water to remove soil particles, was placed in a 150 ml airtight plasma-flask sealed by a rubber stopper. In each
117 flask, air was removed and replaced with a He/C_2H_2 mixture (90/10 v/v) to create anoxic conditions and inhibit
118 N_2O-reductase. Potassium nitrate (50 µg of N-KNO_3 g^{-1} of fresh root or dried soil) was added to each vial to
119 provide microbial communities with nitrate source. The amount of N_2O during incubation at 28 °C was measured
120 each 4 h for 48 h. The slope of the linear regression was used to estimate anaerobic respiration (denitrification
121 enzyme assay without the addition of carbon) to estimate the produced N_2O ($g^{-1}.h^{-1}$). N_2O was measured with a
122 gas chromatograph coupled to a micro-catharometer detector (µGC-R3000, SRA instruments, Marcy l'Etoile,
123 France).

124

125 ***Denitrification enzyme activity (DEA) of plant roots and soils***

126 We measured the denitrification enzyme activity (DEA) in soil, according to Bardon *et al.* (2014). Ten grams of
127 fresh soil sample (RAS from each plant and bulk soil) were placed in a 150 ml airtight plasma-vial sealed with a
128 rubber stopper. In each flask, air was removed and replaced with a mixture of He/C_2H_2 (90/10 v/v) to create anoxic
129 conditions and inhibit N_2O-reductase. A nutritive solution (0.5 ml) containing glucose (0.5 mg of C-glucose.g^{-1} of
130 dried soil), glutamic acid (0.5 mg of C-glutamic acid g^{-1} of dried soil) and potassium nitrate (50 µg of N-KNO_3 g^{-1} of dried soil) was added to the soil. The amount of N_2O during incubation at 28 °C was measured each hour for
132 5 h. The slope of the linear regression was used to estimate anaerobic respiration (denitrification) by measuring

the production of N_2O ($g^{-1}.h^{-1}$) using a gas chromatograph coupled to a micro-catharometer detector (µGC-R3000, SRA instruments, Marcy l'Etoile, France).

RNA extraction and cDNA synthesis

RNA was extracted from the RAS and root system from each plant rhizosphere in triplicates using an RNA "Power Soil isolation" kit (MO BIO) that produced total non-degraded RNA according to the manufacturer's recommendations. DNA was removed from RNA extracted from RAS and root tissues by using RNeasy Mini Kit (Qiagen) according to the manufacturer's recommendations. 16S rRNA gene amplifications (300 bp) were carried out using the treated RNA as a template to ensure that DNA was completely removed. One µg of RNA from each RAS and root system sample retrieved from each plant was transcribed into complementary DNA (cDNA) using the SuperScript III Reverse Transcriptase (Life Technologies) according to the manufacturer's protocol and stored at -20 °C.

nirK *and* **nirS** *genes expression*

Denitrifier abundance was estimated by real-time quantitative reverse transcription PCR (qRT-PCR) targeting the *nirK* and *nirS* genes encoding the copper and cd_1 nitrite reductases, respectively. For *nirK*, the amplification was performed using the primers nirK876 (5'-ATYGGCGGVCAYGGCGA-3') and nirK1040 (5'-GCCTCGATCAGRTTRTGGTT-3') (Henry et al, 2004). The 20 µl final reaction volume contained SYBRgreen PCR Master Mix (QuantiTect SYBRgreen PCR kit, Qiagen, Courtaboeuf, France), 1µM of each primer, 400 ng of T4gp32 (MPbiomedicals, Illkvich, France) and from 1 to 3 µl of cDNA according to 16S rRNA gene normalisation. Thermal cycling was as follow: 15min at 95°C; 6 cycles of 95°C for 15s, 63°C for 30s with a touchdown of -1°C by cycle, 72°C for 30s; 40 cycles of 95°C for 15s, 58°C for 30s and 72°C for 30s. For *nirS*, the amplification was performed using the primers nirSCd3aF (5'-AACGYSAAGGARACSGG-3') and nirSR3cd (5'-GASTTCGGRTGSGTCTTSAYGAA-3') (Throbäck et al, 2004). The 25 µl final reaction volume contained SYBRgreen PCR Master Mix (QuantiTect SYBRgreen PCR kit, Qiagen, Courtaboeuf, France), 1µM of each primer, 400 ng of T4gp32 (MPbiomedicals, Illkvich, France) and from 1 to 3 µl of cDNA according to 16S rRNA gene normalisation. Thermal cycling was as follow: 15min at 95°C; 6 cycles of 95°C for 15s, 59°C for 30s with a touchdown of -1°C by cycle, 72°C for 30s and 80°C for 30s; 40 cycles of 95°C for 15s, 54°C for 30s and 72°C for

30s and 80°C for 30s. The standard curves for *nirK* and *nirS* qPCR were generated by amplifying 10-fold dilutions (10^7 – 10^2) of a linearized plasmid containing the *nirK* gene of *Sinorhizobium meliloti* 1021 and *nirS* gene of *Pseudomonas stutzeri* Zobell DNA (GenArt, Invitrogen, Lifetechnologies, Regensburg, Germany). Melting curves analysis confirmed the specificity of amplification and amplification efficiencies for *nirK* and *nirS* genes were higher than 90%.

Sequencing of 16S rDNA gene

For each plant, 10 µL of cDNA obtained from RNA extracted from the root system and the RAS from each plant in triplicates were sent to FASTERIS (Switzerland) for sequencing using MiSeq Illumina technology. V3-V4 domain of 16S rRNA was amplified with tagged primers 16S Fwd primer 3'-CCTACGGGNGGCWGCAG-5' and 16S Rev primer 3'-GACTACHVGGGTATCTAATCC-5'. Tagged primers structure was 5'- $N_{2\text{-}4}X_6P_n$ -3', with "$N_{2\text{-}4}$" were random bases, "X_6" were 6-bases tag and "P_n" was specific primer. Amplification conditions were 3 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 55 °C, 90 s at 72 °C, and 5 min at 72 °C.

Isotope labelling and analysis of plant and soil material

After 5 weeks of plant growth, all pots (9 pots per plant and 9 pots of bulk soil) received the same amount of two forms of nitrogen: ammonium (NH_4^+) and nitrate (NO_3^-), and where the nitrogen was either ^{14}N or ^{15}N. 3 replicates from each plant and bulk soil treatment received ^{15}N labelled ammonium and unlabelled nitrate ($^{15}NH_4^+$+ NO_3^-) and 3 others replicates received unlabelled ammonium and ^{15}N labelled nitrate (NH_4^+ + $^{15}NO_3^-$). The remaining 3 replicates per plant and bulk soil treatment received unlabelled nitrogen (NH_4^+ + NO_3^-) in order to measure the natural abundance of ^{15}N. The four plant species and the unplanted soil underwent three treatments in triplicates resulting in 45 pots.

In total, 0.24 µg $N.g^{-1}$ dry soil was added with 50 % of each nitrogen form. Five millilitres of each solution were added to each pot in 1 ml aliquots with a single injection homogeneously distributed over the soil surface. Each aliquot was injected with a syringe with 21-gauge needle that was slowly removed to ensure uniform distribution throughout the profile. After 12 h of incubation, the shoots of all pots were cut off and stored at -80 °C. The root systems and their root-adhering soil fractions were subsequently separated. The roots were rinsed with tap water. All roots and RAS samples were stored at -80 °C. The 12 h incubation time was used in accordance with previous

studies, which showed maximal uptake of label around this time (Streeter *et al*., 2000; Bardgett *et al*., 2003; Weigelt *et al*., 2003).

After freeze-drying, 2.5 mg of crushed plant tissue and 10 mg of soil were used to measure nitrogen concentration and the $^{15}N/^{14}N$ ratio using an isotopic ratio mass spectrometer (Isoprime 100, Isoprime Ltd, Manchester, UK) coupled in continuous flow with an elemental analyser (FlashEA 1112 Thermo Electron, Milan, Italy). N concentration data were calibrated using aspartic acid. For the $^{15}N/^{14}N$ measurements, a two-point normalization of the data was performed using international reference material IAEA-305a and IAEA-311 [28]. The $^{15}N/^{14}N$ ratios were expressed as atomic fraction (i.e. atomic %, Coplen, 2011):

$$x(^{15}N) = (^{15}N/^{14}N_{sample}) / (1 + {}^{15}N/^{14}N_{sample})$$

Values of atom fraction and concentration of N (in %) were used to calculate uptake of ^{15}N on a per unit mass basis (mg excess of ^{15}N per gram). Mean values of ^{15}N abundance of the unlabelled control plants were used as reference for ^{15}N excess.

Bioinformatics analysis

Sequence processing and data analysis

Sequence data were processed and analysis of high-throughput community sequencing data was performed with QIIME version 1.8 (Caporaso *et al*., 2010). Sequences were trimmed of barcodes and primers, and then short sequences (< 200 bp), sequences with ambiguous base calls, and sequences with homopolymer runs exceeding 6 bp were removed. Operational Taxonomic Units (OTUs) were then defined by clustering at 97% similarity. Final OTUs were taxonomically classified using Blast (Altschul *et al.*, 1990) and compiled into each taxonomic level into both "counts" and "percentage" files. OTU tables were rarefied at the lowest sequencing depth to control for differences in sequencing depth. A total of 7,185,297 valid reads and 392,892 OTUs were obtained from the 27 samples through sequencing analysis (3 root samples and 3 root-associated soil samples per plant species (4 species) and 3 bulk soil samples).

Alpha diversity analysis was done using the Phyloseq R package version 1.20.0 (McMurdie & Holmes, 2013). Species richness (observed richness) and species evenness (Inverse Simpson index) were estimated by multiple subsampling with replacement ($n = 10^3$) to the minimum libraries sizes to standardize the sequencing effort.

Core microbiota

The core microbiota was identified using QIIME (Caporaso *et al*., 2010) and was determined by plotting OTU abundance in the core at 5% intervals (from 50% to 100% of samples). We defined the core microbiota of each plant as the OTUs present in 100% of samples. Determination of a core microbiota was accomplished by comparing all samples from each plant across the two compartments (RAS and root). Any taxa found to be ubiquitous across all samples were then defined to be part of the core microbiota of the compartment. From these data Venn diagrams were constructed using the R package: VennDiagram, to show common and unique OTUs within the four plant species (*T. aestivum*, *B. napus*, *M. truncatula and A. thaliana*).

Network analysis

Network inference was made by computing all Spearman's rank correlations between all OTUs of the "family" taxonomic level. P-value was adjusted using the procedure of Benjamini and Hochberg (1995) to finally consider only significant correlation (Spearman correlation coefficient $|r| > 0.8$ and P-adj < 0.05). The same procedure was used to compute the correlation between each OTU and each compartment (root system and root-adhering soil). Each network was visualized, analysed and different metrics (Table S1) (i.e. degree, diameter, average path length, average clustering coefficient, modularity) were calculated using the Gephi open-source software (Bastian *et al.*, 2009).

We analysed OTUs interactions by comparing the high and low DAE graphs for the R and RAS compartments. By computing intersection, union or difference of this graphs, we can retrieve edges present or not in our DAE condition of interest. The graphical analysis was done using the igraph R package.

Statistical analysis

All results are presented as means (± standard error). For each plant, a one-way analysis of variance (ANOVA) and post-hoc Tukey HSD were performed to test the effect of root exudation on measured variables. Before analysis, Shapiro and Bartlett tests were performed to ensure conformity with the assumptions of normality and homogeneity of variances. Effects with $p < 0.05$ are referred to as significant.

In order to test the significance between microbiota inhabiting the RAS and those colonizing the root tissues on all plants a non-parametric permutation-based multivariate analysis of variance (PERMANOVA, vegan R

package, Anderson 2001) on abundance based (Bray-Curtis) dissimilarity matrix was performed. All statistical analyses were carried out using R statistical software 3.1.0 (R Development Core Team, 2008).

Data deposition

The sequence data generated in this study was deposited at EMBL-ENA public database (http://www.ebi.ac.uk/ena/data/view/PRJEB25281).

RESULTS

Denitrifying enzyme activity in plant root systems

To investigate the impact of the root exudates produced by each plant species on denitrifying activity, we measured the emission of N_2O after nitrate amendment only on each plant root system, without adding any carbon source (Fig. 1A). The denitrification activity of the microbiota colonizing the root system of each plant species differs significantly from one another. The denitrification activity was higher on the root system of *T. aestivum* and *A. thaliana* (5.2 and 4.6 g $N\text{-}N_2O\ h^{-1}.g^{-1}$ dried roots, respectively) and very low on the root system of *M. truncatula* and *B. napus* (0.6 and 0.08 g $N\text{-}N_2O\ h^{-1}.g^{-1}$ dried roots, respectively). Interestingly, *B. napus* root exudates appear to inhibit denitrification activity in the root system or counterselect denitrifying microorganisms.

Denitrifying enzyme activity on the root-adhering soil

To determine the potential rate of the denitrification of microorganisms present on the root-adhering soil, the DEA of the RAS fractions retrieved from each plant rhizosphere was measured. The DEA of *T. aestivum, B. napus* and *M. truncatula* are significantly different from the unplanted soil (Fig. 1B), indicating a rhizosphere effect. However, the DEA of *A. thaliana* is significantly lower than that of the unplanted soil and the rhizosphere of the other plants. In addition, *T. aestivum, B. napus* and *M. truncatula* do not differ significantly from each other (Fig. 1B).

Preferential uptake of soil nitrogen forms by plant species: NO_3^- and/or NH_4^+

In shoot tissues, the ^{15}N content after the separate addition of NO_3^- or NH_4^+ for each plant species reveals a significant preferential uptake of NO_3^- by *T. aestivum, B. napus* and *A. thaliana* plantlets (Fig. 2A). Conversely, none of the forms of N tested are preferentially absorbed by *M. truncatula* in our experimental conditions. *B. napus* had a significantly greater uptake capacity for NO_3^- than the other plant species. The patterns of the root tissue contents in ^{15}N show the same trend as the shoot tissues with a significant preferential uptake of NO_3^- by *T. aestivum* and *B. napus* (Fig. 2B). As with the shoot tissues, *M. truncatula* uptakes NO_3^- or NH_4^+ forms of nitrogen.

Diversity of active microbiota inhabiting each plant rhizosphere

To investigate the impact of the root exudates produced by each plant species on the selection of active microbiota, the RNA extracted from the root-adhering soil and the root system was reverse transcribed and analysed using 16S rRNA gene sequencing. An average of 7,185,297 sequences were generated and utilized for analysis. The rarefaction curves, displaying the observed OTUs richness as a function of the sequencing effort, indicated that the sequencing depth has almost been reached to completely capture the diversity present in the bulk soil, RAS and root tissues of all the plants (Fig. S1). The richness and diversity of species (Inverted Simpson) were significantly increased in the RAS compared to the bulk soil for each plant, except for *M. truncatula,* and decreased in the root compartment compared to the RAS fraction, especially for *A. thaliana* and *B. napus* (Fig. S2).

Several phyla were present in different amounts between the two compartments studied (RAS and root system) and between the plants (Fig. 3A). In addition, globally on all plants, significant differences (p. value = 0.001) were observed between microbiota inhabiting the RAS and those colonising the root system. The general trend is a very low difference between microbial diversity in the RAS fraction of all the plantlets compared to the one of the reservoir (bulk soil) at the phyla level. Another general trend is an important increase in the *Bacteroidetes* abundance (five-fold in the rhizosphere of *T. aestivum*, *B. napus* and *M. truncatula*) and of the OD1 phylum (from x15 to x150 in the four rhizosphere samples) (Additional File 1). In the root tissues, *Chloroflexi* was more abundant on the root system of *T. aestivum* (8%) than the other plants (3-5%), while *Bacteroidetes* was more abundant on the root system of *B. napus* (15%) and *T. aestivum* (13%) compared to the other plants (9-10%) (Fig. 3A, Additional File 1). *Verrucomicrobia* was highly enriched on the root system of *B. napus* (8%) compared to the other plants (1-3%). *Firmicutes* and *Acidobacteria* were less abundant on the plant root systems (1-2% and 1-3%

296 respectively), except on the root tissues of *A. thaliana* where they were slightly more abundant (3% and 5%
297 respectively) (Fig. 3A, Additional File 1). The relative abundance of *Crenarchaeota* was increased on the root
298 system of *A. thaliana* (0.3%) compared to that of the other plants (less than 0.07%). For the RAS fractions, few
299 differences in phyla abundance were observed between the plant species. However, *Bacteroidetes* was more
300 abundant in the RAS of *A. thaliana* (6%) than in those of *T. aestivum, B. napus* and *M. truncatula* (2-3%).
301 *Planctomycetes* was counter-selected in the RAS fraction of *A. thaliana* (9%) compared to other plant rhizospheres
302 and bulk soil (15-17%) (Fig. 3A, Additional File 1).

303 Some phyla have been more enriched on the RAS than on the root tissues and *vice versa* independently of the
304 plant species (Fig. 3A, Additional File 1). For example, this is the case for *Actinobacteria, Gemmatimonadetes,*
305 *Plantomycetes* and *Crenarchaeota,* which were more abundant in the RAS of *B. napus*, *T. aestivum* and *M.*
306 *truncatula* compared to their root systems (from two-fold to ten-fold increases). Despite the high abundance of
307 *Proteobacteria* in all the plant rhizospheres, they were more abundant on the root tissues (44-52%) than in the
308 RAS (34-39%).

309 To examine a potential role of the plant species on the root-associating bacterial assemblage, we compared the
310 bacterial profiles obtained from *T. aestivum, B. napus, M. truncatula* and *A. thaliana* root compartments (Fig. 3B).
311 Within the root OTUs (rOTUs) community, only four OTUs were enriched in the root systems of all the plants
312 studied, while 21, 106, 74 and 103 OTUs were significantly enriched in the root systems of *A. thaliana*, *M.*
313 *truncatula*, *T. aestivum*, and *B. napus*, respectively (Fig. 3B, Additional File 2). The common and unique OTUs
314 retrieved from the root system of each plant are shown in Additional File 2.

315 To examine the impact of the plant species on shaping the bacterial community inhabiting the root-adhering soil
316 (RAS), we compared the bacterial profiles obtained in the root-adhering soil of *T. aestivum*, *B. napus*, *M.*
317 *truncatula* and *A. thaliana* (Fig. 3C). We identified 24 root-adhering soil OTUs (rasOTUs) shared by the four plant
318 species. These shared OTUs can be regarded as the core microbiome of the four rhizospheres. Unique rasOTUs
319 corresponding to the bacterial community specifically inhabiting each plant species were also observed with 130,
320 65, 133 and 1683 OTUs found in the rhizosphere of *A. thaliana*, *M. truncatula*, *T. aestivum*, and *B. napus*,
321 respectively. Common and unique OTUs retrieved from the plant root-adhering soils are shown in Additional File
322 3.

323

Network description

To explore co-occurrences between the bacterial phyla colonizing the root system and inhabiting the rhizosphere (RAS fractions) of *T. aestivum, B. napus, M. truncatula* and *A. thaliana*, we used a network inference based on strong and significant correlations (using non-parametric Spearman's) (Fig. 4, Table S1). The number of positive correlations (co-occurrences) was higher than the number of negative correlations (co-exclusions) in the rhizosphere of *B. napus* and *A. thaliana* and equal for the two others (*T. aestivum*, *M. truncatula*) (Table S1).

The bacterial network of OTUs from the root system (rOTU) and the RAS (rasOTUs) compartments of *B. napus* is highly connected (Fig. 4). The structure of the network demonstrates densely connected groups of nodes, forming a clustered topology. Comparing the properties of the calculated networks, we observed that the *B. napus* network contains a significantly higher percentage of negative (220) and positive (171) edges than the other plants (Table S1). OTUs considered to be keystone species belonged primarily to different genera within the phyla of *Proteobacteria*, *Planctomycetes* and *Actinobacteria* (Fig. 4).

The *T. aestivum* bacterial network that associates OTUs from the root system (rOTU) and its RAS (rasOTUs) is also highly connected (Fig. 4) with 105 positive edges and 110 negative edges (Table S1). The OTUs considered to be keystone species (depicted as nodes with larger sizes in the network) belonged primarily to different genera within the phyla of *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* (Fig. 4).

For the bacterial networks of *M. truncatula* and *A. thaliana*, we noticed a clear separation between the rOTUs and rasOTUs with a very weak connection between them. More bacterial phyla colonizing RAS co-occur in the rhizosphere of *M. truncatula* compared to the root system (Fig. 4). For *A. thaliana,* the co-occurring bacterial phyla display an equal distribution in the RAS and the root system, and very few co-occur on the root tissues.

Finally, co-exclusions and co-occurrences found between certain rOTUs and/or rasOTUs retrieved from each plant are shown in Additional File 4. Overall, regardless of the plant studied, we noticed more co-exclusion and less co-occurrence relationships among the significant interactions (Fig. 4 and Table S1). Among several positive interactions observed, we noted strong co-occurrence relationships for some members of *Firmicutes* (*Paenibacillaceae*) with *Acidobacteria* and *Actinobacteria* (*Frankiaceae*) in the RAS of *B. napus* (Additional File 4). A positive correlation was also observed between *Nitrospiraceae* and *Actinobacteria* in the RAS of *B. napus* and *A. thaliana* and between *Nitrospiraceae* and *Planctomycetes* in the RAS of *T. aestivum, B. napus* and *A. thaliana.* A positive correlation was also observed between *Rhizobiaceae* and *Chloroflexi* and between

352 *Chlamydiales* and *Planctomycetales* on the root system of *T. aestivum* and *M. truncatula,* respectively (Additional
353 File 4).

354 Among several negative interactions observed, we noted a co-exclusion relationship for *A. thaliana* for the OTUs
355 of the *Acidobacteria* (*Chloracidobacteria*) found on the RAS with the OTUs of the *Firmicutes* (*Paenibacillaceae*)
356 found on the root system (Additional File 4). In the rhizosphere of *T. aestivum*, the OTUs from *Gemmatimonadetes*
357 were negatively correlated with those of the *Opitutales* (*Verrucomicrobia*). For example, in the rhizosphere of *B.*
358 *napus*, the OTUs of the *Chloroflexi* correlate negatively with the OTUs of the *Comamonadaceae*.

359 By comparing OTUs interactions under high and low DEA conditions in the R and RAS compartments of the
360 studied plant, we have identified a few OTUs that co-occur only in the RAS of *T. aestivum* and *A. thaliana,* with
361 high DEA, while, other OTUs co-occur only in the R of *T. aestivum* and *B. napus* with high DEA (Additional File
362 5), suggesting a potential role for these OTUs in denitrification. This is the case, for example, of the co-occurrence
363 on the RAS of *T. aestivum* and *B. napus* between OTUs of *Nitrospira* and *Solirubrobacterales* and
364 *Rhodospirillales* and *Candidatus nitrososphaera* or between OTUs of *Acidobacteria* and *Verrucomicrobia* on the
365 R of *T. aestivum* and *A. thaliana* (Additional File 5).

366 **_nirS_ and _nirK_ transcript levels in the rhizosphere microbiota**

367 To gain additional insights on the role of root exudates in regulating denitrification, we measured the expression
368 of the *nirK* and *nirS* genes on the root system and in the root-adhering soil of each plant species (Fig. 5). The
369 number of *nirK* (encoding a copper-containing nitrite reductase) transcripts from the denitrifiers was higher on the
370 root system of *A. thaliana* and *T. aestivum* (2.3×10^4 and 2.8×10^4 *nirK* cDNA copies per g of dried root
371 respectively), in contrast with a lower expression on the root system of *B. napus* (0.9×10^4 *nirK* cDNA copies per
372 g of dried root) and the almost complete absence of *nirK* expression on the root system of *M. truncatula* (Fig. 5A).
373 The *nirS* gene that encodes a cytochrome cd1-containing nitrite reductase was detected only on the root system of
374 *M. truncatula* (1.7×10^4 *nirS* cDNA copies per g of dried root).

375 Given the level of expression of the *nirK* gene in the root-adhering soil, no significant difference was found
376 between the RAS of *T. aestivum* and *M. truncatula* compared to the bulk soil. In contrast, the expression of *nirK*
377 was significantly decreased in the RAS of *A. thaliana* and *B. napus* compared to the bulk soil (Fig. 5B). As on the
378 root system, the expression of *nirK* is the lowest in the RAS of the *B. napus* plantlets (Fig. 5A and 5B). No
379 expression of *nirS* was detected in the RAS fraction of the four plant species and in the bulk soil. In summary,

only *nirK* (not *nirS*) was expressed on the root system of *T. aestivum* and *A. thaliana* and, to a lesser extent, on the one of *B. napus* and repressed in the presence of *M. truncatula*. *nirK* was repressed in the RAS of *A. thaliana* and *B. napus* and unchanged in that of *T. aestivum* and *M. truncatula*. Surprisingly, *nirS* expression was detected only on the root system of *M. truncatula*.

DISCUSSION

Plant species shape denitrification activity through root exudation

The measurement of the denitrification activity of the root system without additional carbon (Fig. 1) as conducted in a previous study (Guyonnet *et al.,* 2017), likely displayed higher activity than in the root-adhering soil where carbon source was added. This result suggests the role of the root exudates of each plant species in the management of the denitrification activity. This plant intervention might be conducted by selecting beneficial microorganisms involved in plant nutrition and protection against pathogens, which may happen to denitrify. The host plant may also produce compounds that could control the expression of the genes involved in denitrification. Figure 1 shows a higher denitrification activity on the root system of *T. aestivum* and *A. thaliana,* which is consistent with the high expression of the bacterial *nirK* gene for these two host species (Fig. 5). This suggests a potential positive effect of the root exudates from *T. aestivum* and *A. thaliana* to select bacterial populations able to denitrify or to activate the expression of denitrification genes. The very low level of denitrification activity on the root system of *B. napus* is consistent with a lower level of expression of the *nirK* gene compared to that of the root system of *T. aestivum* and *A. thaliana*. These results suggest that the plantlets of *B. napus* may not recruit bacterial populations able to denitrify or may alter the expression of denitrification genes on the root system probably via root exudation. In addition, by measuring the preferential uptake of *B. napus* for nitrate and ammonium, our results demonstrated a greater avidity of *B. napus* for nitrogen uptake primarily in the form of nitrate compared to the other plants (Fig. 2), suggesting potential competition for nitrate with denitrifiers as previously observed for other plants (Kuzyakov & Xu, 2013; Bardon *et al.,* 2014). Denitrification inhibition, named Biological Denitrification Inhibition (BDI), has already been observed in *Fallopia sp.* and has been defined as the ability of the plant to release secondary metabolites such as procyanidins that inhibit denitrifiers, and therefore enables the plant to recover nitrate for its growth (Bardon *et al.,* 2014, 2016, 2017). *B. napus* may perform BDI, because the presence of procyanidins has already been demonstrated on its roots (Wronka *et al.,* 1994, Nesi *et al*., 2009).

On the root system of *M. truncatula*, the nitrogen uptake was very low regardless of its form, suggesting that unlike *B. napus*, competition for nitrogen with denitrifiers is not expected. However, the low denitrification rate primarily achieved by denitrifying bacteria harbouring the *nirS* gene indicates a counter-selection of the populations harbouring the *nirK* gene. However, we did find that a high level of denitrification activity on the legume RAS positively correlated with the *nirK* gene expression (Fig. 1). Several studies have investigated the effect of legume cultivation on nitrogen cycle processes, since legumes associated with nitrogen-fixing bacteria can be used as a substitute for mineral fertilizers (Philippot *et al*., 2007). Researchers reported high denitrification rates with legume rhizospheres compared to other plants (Svensson *et al.*, 1991; Kilian and Werner, 1996) which is consistent with our data. Although, several studies have reported that denitrification is very common in rhizobia and that many strains can denitrify both as nodule bacteroids and as free-living bacteria (Philippot *et al.,* 2007). In our study, either *M. truncatula* root-associated *Rhizobia* (bacteroids or free-living bacteria) are not involved in the denitrification process, since *nirK* gene expression was not detected (Fig. 5, Additional File 2), or it can be assumed that only the *nirS* copy is transcribed, since it was recently suggested that certain rhizobia may possess Cu-type (*nirK*) and cd1-type (*nirS*) nitrite reductase genes (Sánchez and Minamisawa, 2018).

Our results provided evidence that the denitrification activity of the microbial communities inhabiting the rhizosphere (root-adhering soil) of *T. aestivum* was significantly higher than that of the unplanted soil, where less carbon is available (Fig. 1). Similarly, the measurement of the denitrification rate of cereals, such as barley grown in pots (Klemedtsson *et al*., 1987, Metz *et al.,* 2003) and maize in field conditions (Mahmood *et al.,* 1997), also revealed high denitrification activity compared to the unplanted soil. However, even though denitrification is primarily conducted by bacteria, we cannot exclude the role of fungi and archaea as previous studies have already shown their capacity to denitrify (Philippot, 2002a, Maeda *et al*., 2015). In addition, the *nirK* and *nirS* primers used in this study are not universal enough to survey all the soil denitrifiers, since they were designed based on a limited number of sequences, primarily from laboratory strains. Further studies developing new *nirK* and *nirS* primers and targeting denitrification genes expression from fungi and *Archaea* are needed to confirm our hypothesis.

Core and specific active microbiota among plant species

Metabarcoding of 16S rRNA revealed the enrichment of members of *Chloroflexi* on the root system of *T. aestivum, Bacteroidetes* and *Verrucomicrobia* on that of *B. napus* and *Firmicute* and *Acidobacteria* on that of *A. thaliana* at

437 the root system level (Figure 3A, Additional File 1). These results are consistent with previous data using a stable-
438 isotope probing (SIP) approach on *B. napus* (Gkarmiri *et al.,* 2017), *A. thaliana* (Bulgarelli *et al*., 2012; Lundberg
439 *et al*., 2012) and wheat (Wang *et al.,* 2016).

440 Comparison of the microbiota inhabiting the root-adhering soil revealed differences in the phyla abundance with
441 high abundance, for example, *Bacteroidetes* in the rhizosphere of *A. thaliana* (Figure 3A, Additional File 1), which
442 is consistent with previous research by Bulgarelli *et al*. (2012) on this model plant. Remarkably, the rhizosphere
443 of *A. thaliana* was enriched with the *Crenarchaeota* phylum, which is consistent with the results of Bressan *et al.*
444 (2009). This phylum, considered to be the most abundant ammonia-oxidizing phylum in soil ecosystems
445 (Leininger *et al*., 2006), has been found to be involved in root exudate assimilation in the rhizosphere of *A. thaliana*
446 using an SIP approach (Bressan *et al.,* 2009).

447 The comparison of the microbial assemblages revealed differences and similarities in the composition of the
448 microbiota inhabiting the roots and the rhizosphere retrieved from the four plant species (Fig. 3B and C). First, we
449 demonstrated that four rOTUs belonging to members of the families *Caulobacteraceae*, *Comamonadaceae*,
450 *Chitinophagaceae* and *Bacillaceae* were enriched in the root systems of all the plants studied. These rOTUs can
451 be considered generalists, since they are associated with the root system of all plants studied as determined by
452 Haichar *et al.* (2008). Second, some clear differences were observed between the microbiota from the four plant
453 root systems (Fig. 3B and C). For example, some members of the *Cytophagaceae* and the *Chitinophagaceae* family
454 are specifically selected by *A. thaliana* (Additional File 3), while members of the families *Opitutaceae*,
455 *Verrucomicrobiacea, Solibacterales* and Ellin 6075 were specifically associated with *B. napus*. In contrast, the
456 enrichment of the members of the *Microbacteriaceae* family appears to be a distinct feature of the microbiota of
457 the *T. aestivum* roots. In a recent study by Tomasek *et al.* (2017), a positive correlation between the abundance of
458 *Cytophagaceae, Chitinophagaceae* and *Microbacteriaceae* and denitrification activity was observed, suggesting
459 that these families potentially play an important role in denitrification at the root of *A. thaliana* and *T. aestivum.*

460 For microbiota colonizing the root-adhering soil, we identified 24 rasOTUs common to all the plants studied
461 (Additional File 3), suggesting that these OTUs represent generalist bacteria, while those retrieved only from a
462 single plant species are considered to be specialist bacteria (Haichar *et al.,* 2008).

463 By comparing the root microbiota with the RAS microbiota, it appears that the root compartment is more selective
464 than the soil surrounding the root system, indicating the specific recognition and nutritional selection of bacterial

communities on the root tissues prior to the release of nutrients into the rhizosphere (Haichar *et al.,* 2008, 2012, 2013).

As the bacterial community colonizing the root system differs according to the plant species, the differences in denitrification activity could also be linked to the selection of certain denitrifying bacteria that are more or less effective for each plant through root exudates. Similarly, the diversity of the bacterial community inhabiting the root-adhering soil could also explain the denitrification activity profiles. Futures studies targeting the diversity of denitrifiers using denitrification genes, such as *nirK* and *nirS,* are needed. As described above, the current challenge is to design optimal *nirK* and *nirS* universal primer pairs to target entire denitrifier communities in the environment as already suggested by Bonilla-Rosso *et al.* (2016).

Microbial interaction networks

Positive correlations between microbial populations suggest the occurrence of a mutualistic interaction and co-existence, while negative correlations might suggest the presence of host-competitive exclusion or a predation relationship between the microorganisms (Steele *et al*., 2011, Barbéran *et al.,* 2011, Ju and Zhang, 2015). A closer look at the bacterial networks shows that several associations confirm or reveal interesting ecological patterns for the taxa that have not been as well studied as was also shown by Barbéran *et al.* (2011) (Fig. 4, Additional File 4). This is the case for the *Crenarchaeotal* OTU, which is particularly remarkable because of our poor understanding of the ecological niches occupied by this taxon, even though it has been proposed that related *Crenarchaeota* are ubiquitous in the soil (Bates *et al*., 2010; Barbéran *et al.,* 2011) and that they may have an important role in the nitrogen cycle as ammonia oxidizers (Simon *et al*., 2000; Leininger *et al*., 2006; Barbéran *et al.,* 2011). In this study, taxa related to *Crenarchaeota* OTUs co-occurred with the *Alphaproteobacteria* class (Ellin329 order) and *Acidobacteria* in the RAS of *B. napus* and with *Chloroflexi* (*Anaerollinea* class) in the RAS of *M. truncatula.* The co-occurrence between the *Crenarchaeota* OTUs is probably involved in the nitrogen cycling with the OTUs of the families *Acidobacteria, Alphaproteobacteria* and *Chloroflexi*, which are probably also involved in carbon cycling (Ward *et al*., 2009; Hug *et al*., 2013; Brauer *et al*., 2016), highlight the link between these two cycles and the importance of each for a better understanding of the other.

It is interesting to note that *Nitrospirales* and *Solirubrobacterales* co-occur only in the RAS of *T. aestivum* and *B. napus* displaying high DEA, suggesting a syntrophic relationship, in which ammonium released by

493 *Solirubrobacterales* during the organic metabolism of N (Tu *et al.,* 2017) could serve as a substrate for *Nitrospira*
494 to achieve nitrification (Daims *et al.,* 2015) and provide nitrate to denitrifiers. Similar interactions might also be
495 suggested between *Candidatus nitrosphaera* participating to nitrification process (Spang *et al.,* 2012) and
496 *Rhodospirillales* known to perform denitrification as described by Saarenheimo *et al.* (2015).

497

498 CONCLUSION

499 Collectively our results have shown that plant species shape denitrification activity and modulate the diversity of
500 active microbiota through root exudation. As expected, a positive effect of the root exudates on denitrification
501 activity was observed on the root system of *A. thaliana* and *T. aestivum,* while *B. napus* appears to alter
502 denitrification activity on the root system through root exudates. This denitrification inhibition is probably due to
503 competition between *B. napus* and the denitrifiers for nitrate, since nitrate is preferentially utilized by *B. napus*.
504 Some OTUs were associated with all the plants studied and are considered generalists, while others are specifically
505 associated with plant species and are considered to be specialists. Network analysis indicated that *Nitrospirales*
506 and *Solirubrobacterales* were positively correlated with high denitrification activity in the RAS of *T.*
507 *aestivum* and *B. napus*, while a positive correlation was observed between *Cytophagaceae,*
508 *Chitinophagaceae* and *Microbacteriaceae* and a high DEA at the roots of *A. thaliana* and *T. aestivum*.

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510

511 ACKNOWLEDGEMENTS

512 This work was supported by the French national programme « EC2CO-MicrobiEn, CNRS », (RhizoDen Project).
513 We thank Elise Lacroix for her help in plant culture and harvesting. Plant culture was performed at the greenhouse
514 platform (FR41, University Lyon1). We thank J. Gervaix for his help on nitrogen form uptake experiments. The
515 authors gratefully thank the editor Prof. Wietse de Boer and reviewers for improving our manuscript.

516

517 CONFLICT OF INTEREST

518 The authors declare no conflict of interest.

519

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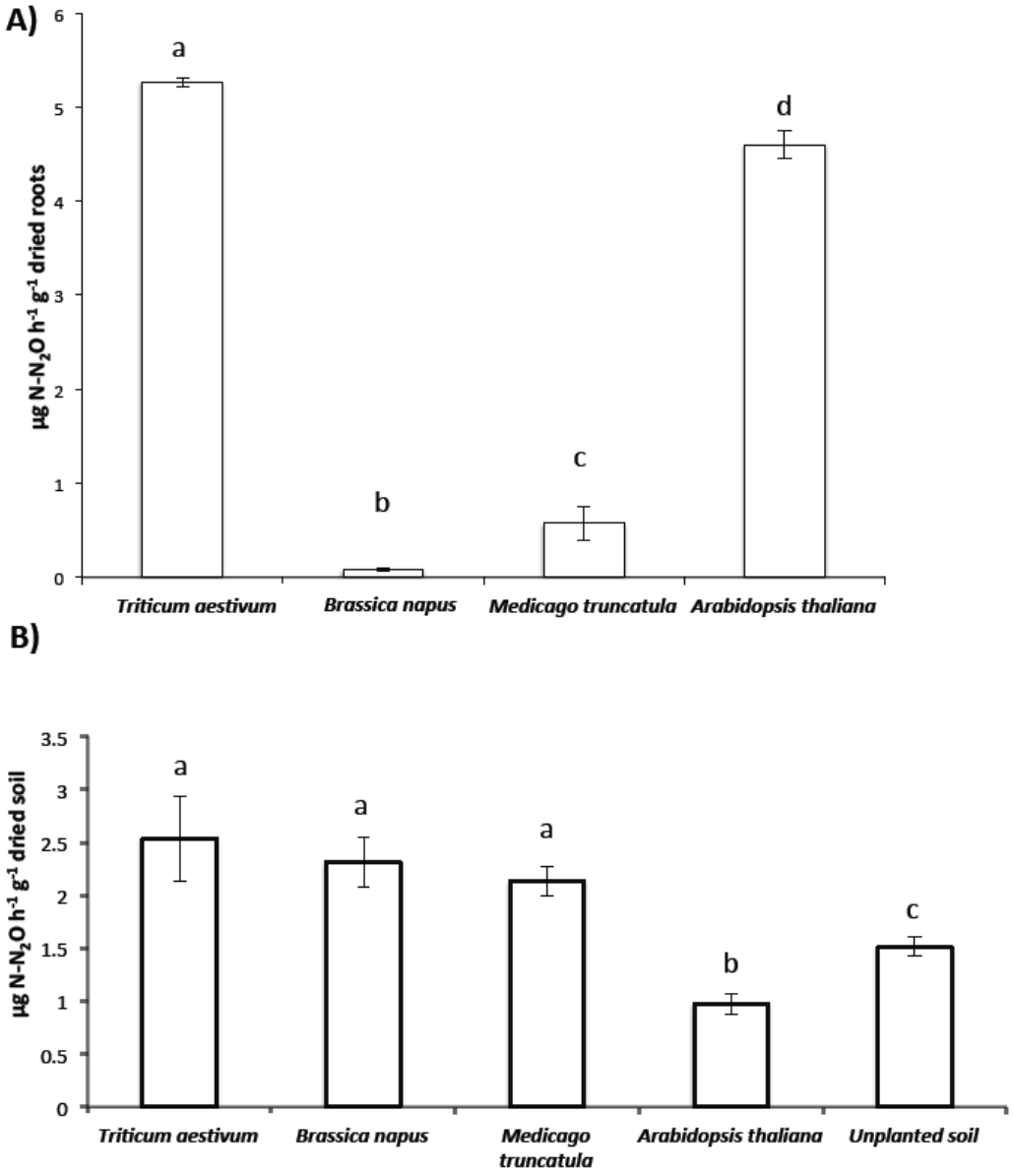

Figure 1

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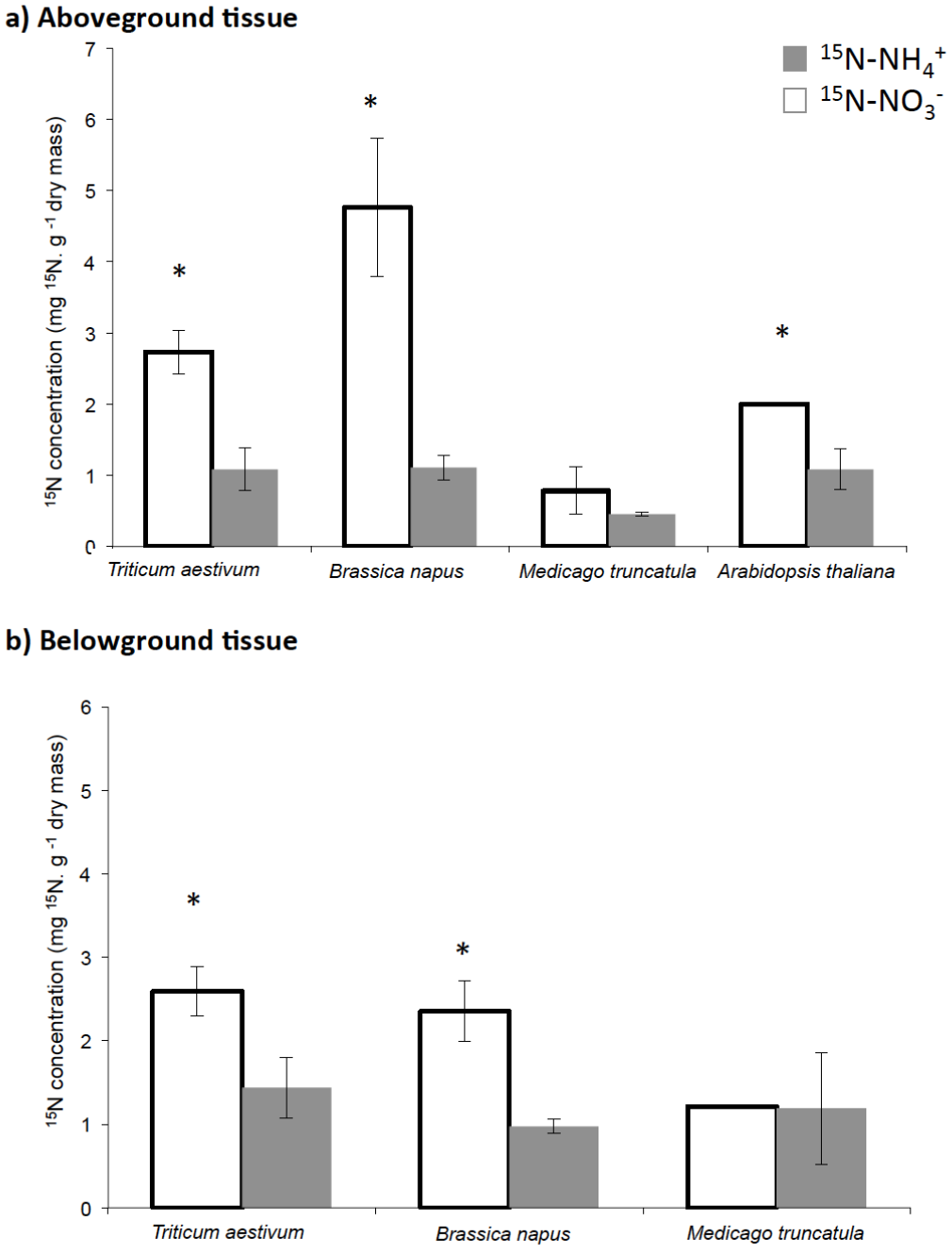

Figure 2

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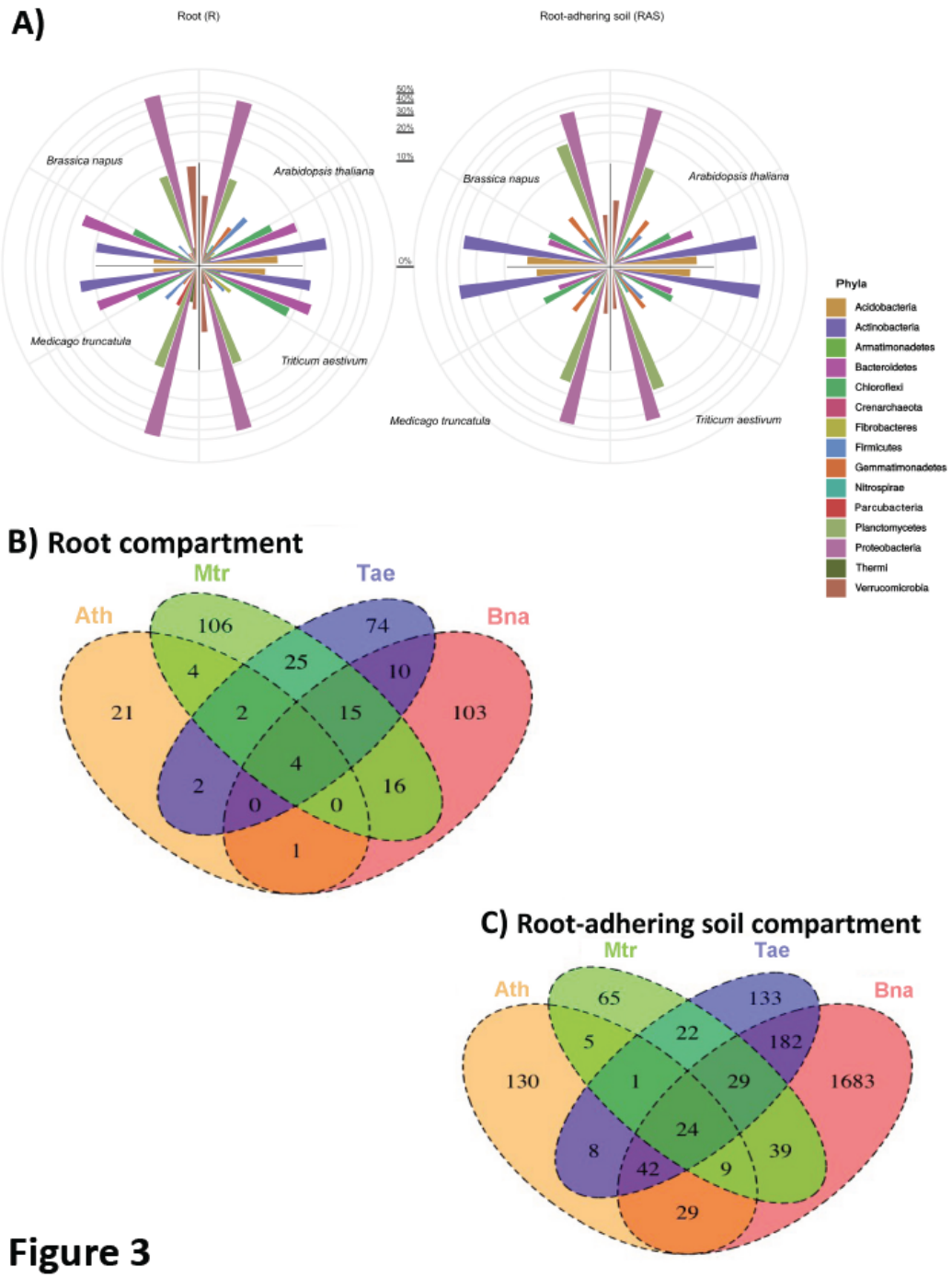

Figure 3

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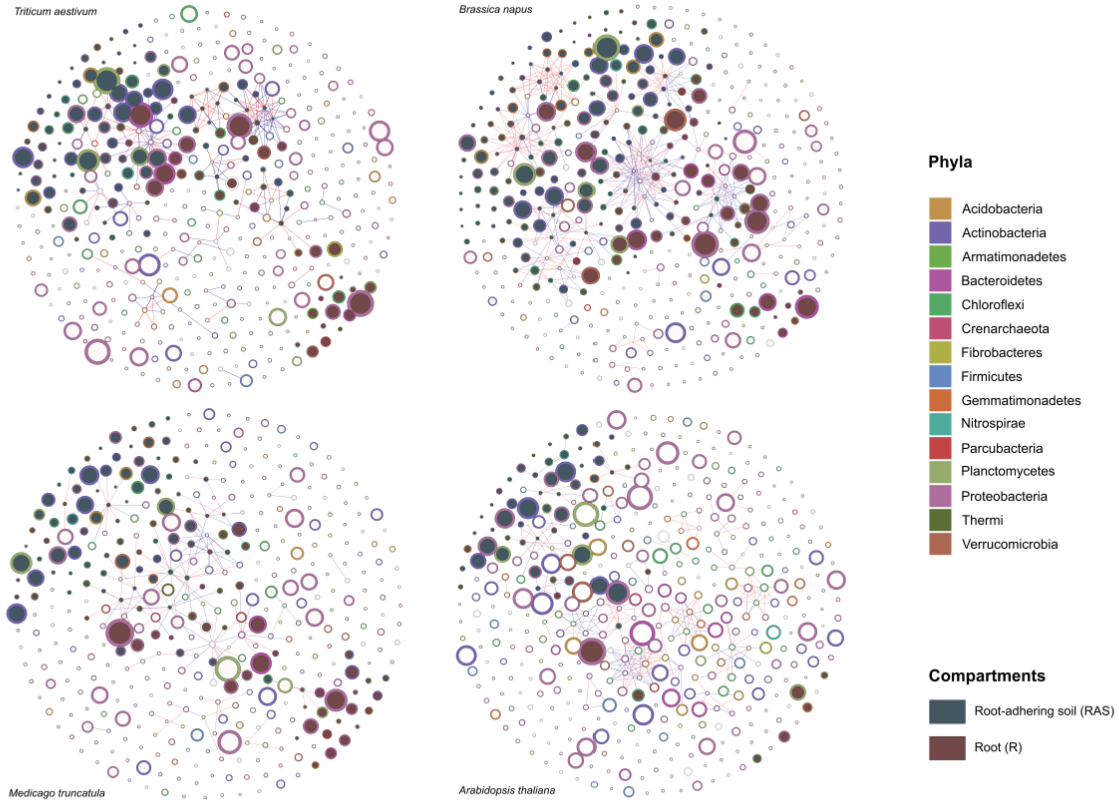

Figure 4

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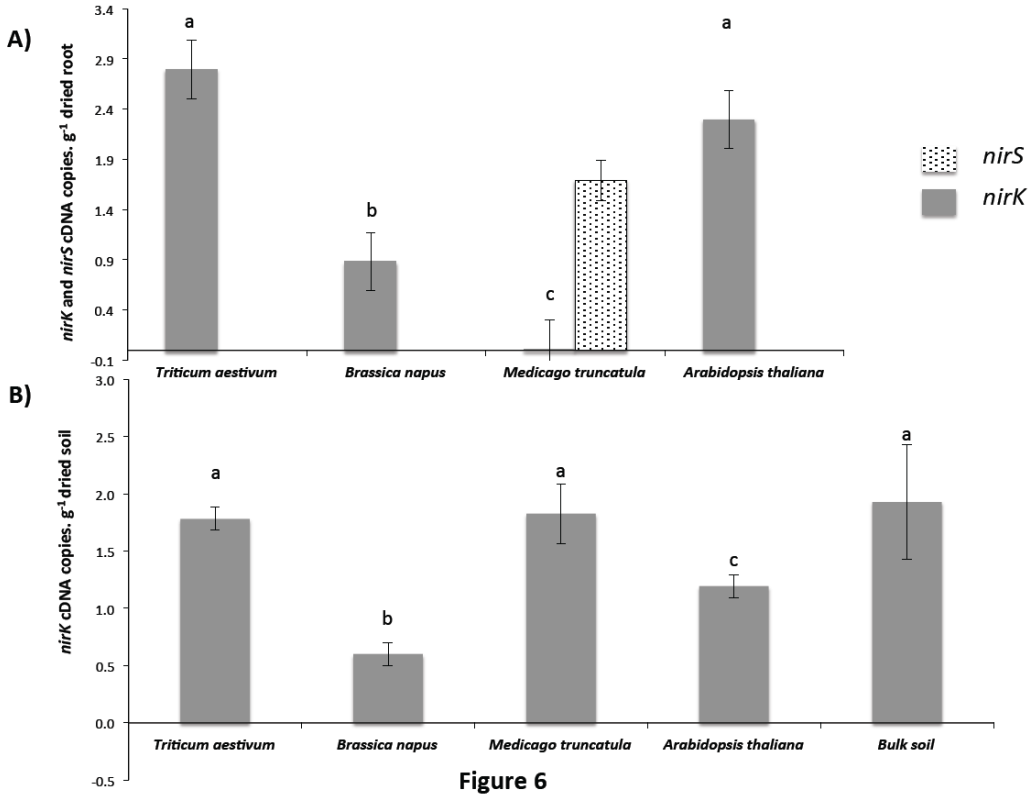

Figure 6

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706 **FIGURES, TABLE, ADDITIONAL FILES**

707 **Figure 1.** Denitrification activities of microbiota colonising the root system and inhabiting the root-adhering soil
708 of wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), barrel clover (*Medicago truncatula*), and *Arabidopsis*
709 *thaliana* plantlets. **(A)** Impact of root exudates on denitrifying activity of microbial community colonising the root
710 system. Denitrification activity (g $N\text{-}N_2O\ h^{-1}.g^{-1}$ dried roots) was measured in triplicates after the addition of nitrate
711 source only. **(B)** Denitrification enzyme activity (DEA) of soil samples amended with nitrate (50 µg of $N\text{-}KNO_3$
712 g^{-1} of dried soil) and carbon sources (0.5 mg of C-glucose and 0.5 mg of C-glutamic acid g^{-1} of dried soil) measured
713 in triplicates. Letters show which means differed between treatments (Tukey's test; $\alpha = 0.05$). Vertical bars: Means
714 ± Standard Errors.

715

716 **Figure 2.** Nitrogen uptake of ammonium and nitrate for aboveground **(A)** and belowground **(B)** tissues 12 h after
717 ^{15}N labelling (NO_3^- or NH_4^+) of wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), barrel clover (*Medicago*
718 *truncatula*), and *Arabidopsis thaliana* plantlets. Vertical bars: Means ± Standard Errors. Means significantly
719 different from $^{15}N\text{-}NO_3^-$: *, $P < 0.05$. Nitrogen uptake was not measured belowground for *A. thaliana* due to low
720 amount of roots.

721

722 **Figure 3.** Analysis of bacterial diversity. **(A)** Distribution of the 15th majors bacterial phyla (abundance in %)
723 among the root system and the root-adhering soil of wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), barrel
724 clover (*Medicago truncatula*), and *Arabidopsis thaliana* plantlets. A Venn diagram showing shared and unique
725 bacterial OTUs at 100% identity among bacterial community colonising the root system **(B)** and those inhabiting
726 the root-adhering soil **(C)** retrieved from the rhizosphere of wheat (*Triticum aestivum*), rapeseed (*Brassica napus*),
727 barrel clover (*Medicago truncatula*), and *Arabidopsis thaliana*.

728

729 **Figure 4.** Significant co-occurrence and co-exclusion relationships among the microbiota inhabiting the root-
730 adhering soil (in green) and colonising the root system (in brown) of wheat (*Triticum aestivum*), rapeseed
731 (*Brassica napus*), barrel clover (*Medicago truncatula*), and *Arabidopsis thaliana* plantlets. The colour of nodes
732 (ring of the cercal) corresponds to the phylum, while the size of the nodes is proportional to their abundance. The

red and blue lines specify significant positive and negative correlations (Spearman correlations ≥ 0.8 or ≤-0.8, *P.adj* < 0.05) between two nodes, respectively. Full circles with green or brown colour correspondent to OTUs more abundant on the root system and the root-adhering soil respectively. Empty circles correspondent to OTUs with the same abundance between the root system and the root-adhering soil compartments.

Figure 5. Predictive functional metagenome composition. Heatmap of the normalized relative abundances of the predicted functional categories (level 3) of the microbiota colonising the bulk soil, the root system and the root-adhering soil (RAS) of wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), barrel clover (*Medicago truncatula*) and *Arabidopsis thaliana* plantlets.

Figure 6. Quantification of *nirK* and *nirS* genes transcripts from RNA extracted from the root system (A) and the root-adhering soil (B) from wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), barrel clover (*Medicago truncatula*) and *Arabidopsis thaliana* plantlets by real-time RT-PCR. Letters show which means differed between treatments (Tukey's test; $\alpha = 0.05$). Vertical bars: Means ± Standard Errors.

Additional files:

Table S1. Global network statistics for bacterial association networks from the rhizosphere of wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), barrel clover (*Medicago truncatula*), and *Arabidopsis thaliana*. Red edges correspondent to positive correlations (co-occurrences) and blue one to negative correlations (co-exclusions).

Figure S1: Rarefaction curves demonstrating species richness of microbiota retrieved from the root system (R) and root-adhering soil (RAS) compartment from wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), barrel clover (*Medicago truncatula*), and *Arabidopsis thaliana* plantlets.

Figure S2: Species Richness and diversity (Inverted Simpson) of microbiota associated with the bulk soil (BS), the root-adhering soil (RAS) and the root system (R) of wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), barrel clover (*Medicago truncatula*), and *Arabidopsis thaliana* plantlets.

Additional File 1: Abundance in percentage (%) of the 15 more abundant phyla (used in Figure 3) retrieved from the bulk soil and the root system (R) and the root-adhering soil (RAS) of wheat (*Triticum aestivum*), rapeseed (*Brassica napus*), barrel clover (*Medicago truncatula*), and *Arabidopsis thaliana* plantlets.

Additional File 2: Taxonomic affiliation of OTUs retrieved from root tissues **(tab 1)** shared by all the plants (*Triticum aestivum*/Tae, *Brassica napus*/Bna, *Medicago truncatula*/Mtr, and *Arabidopsis thaliana*/Ath) and representing the core microbiome; (**tab 2)** present in Ath only; (**tab 3)** present in Bna only; **(tab 4)** present in Mtr only and **(tab 5)** present in Tae only.

Additional File 3: Taxonomic affiliation of OTUs retrieved from the root-adhering soil **(tab 1)** shared by all the plants (*Triticum aestivum*/Tae, *Brassica napus*/Bna, *Medicago truncatula*/Mtr, and *Arabidopsis thaliana*/Ath) and representing the core microbiome; (**tab 2)** present in Ath only; (**tab 3)** present in Bna only; **(tab 4)** present in Mtr only and **(tab 5)** present in Tae only.

Additional File 4: Taxonomy of OTUs (nodes in Fig. 4) and their significant (spearman value indicated) co-exclusion/co-occurrent OTUs retrieved from the root system (R) or the root-adhering soil (RAS) of *Brassica napus* (**tab 1)**, *Arabidopisis thaliana* **(tab 2)***, Triticum aestivum* **(tab 3)** and *Medicago truncatula* **(tab 4).**

Additional File 5 : OTUs interactions associated only with high DAE conditions from the root system (R) and the root-adhering soil (RAS) compartments. OTUs co-occurring only in the RAS of *Triticum aestivum* and *Arabidopsis thaliana* presenting high DEA. OTUs co-occurring only in the R of *Triticum aestivum* and *Brassica napus* presenting high DEA.

Table S1.

	Triticum aestivum	*Brassica napus*	*Medicago truncatula*	*Arabidopisis thaliana*
Nodes	436	447	406	429
Edges (red for co-occurrence)	110	220	71	151
Edges (blue for co-exclusions)	105	171	60	104
Degree	0.98	1.749	0.645	1.189
Average path length	2.247	2.352	2.062	1.909
Diameter	6	6	4	6
Clustering coef	0	0	0	0
Modularity	0.853	0.851	0.895	0.904

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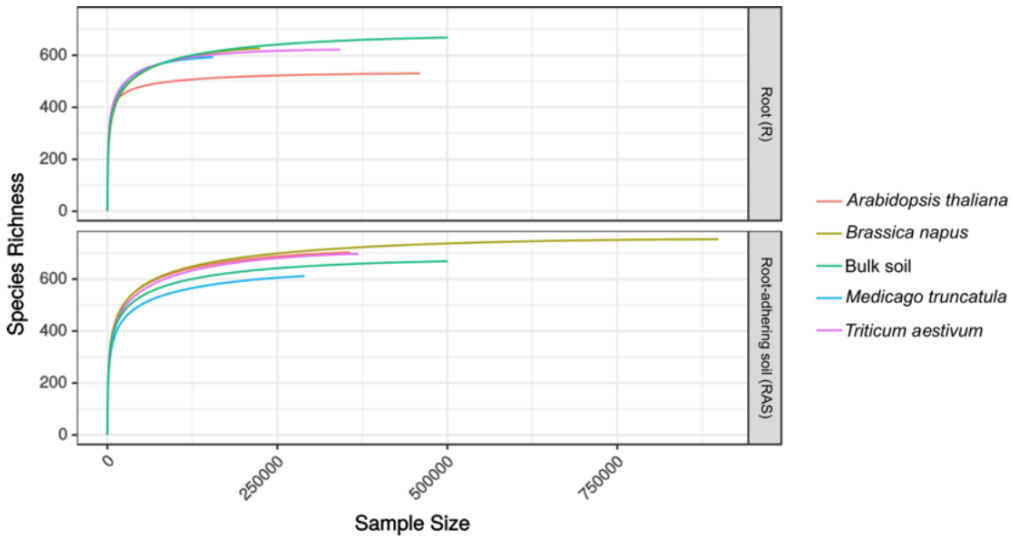

Figure S1

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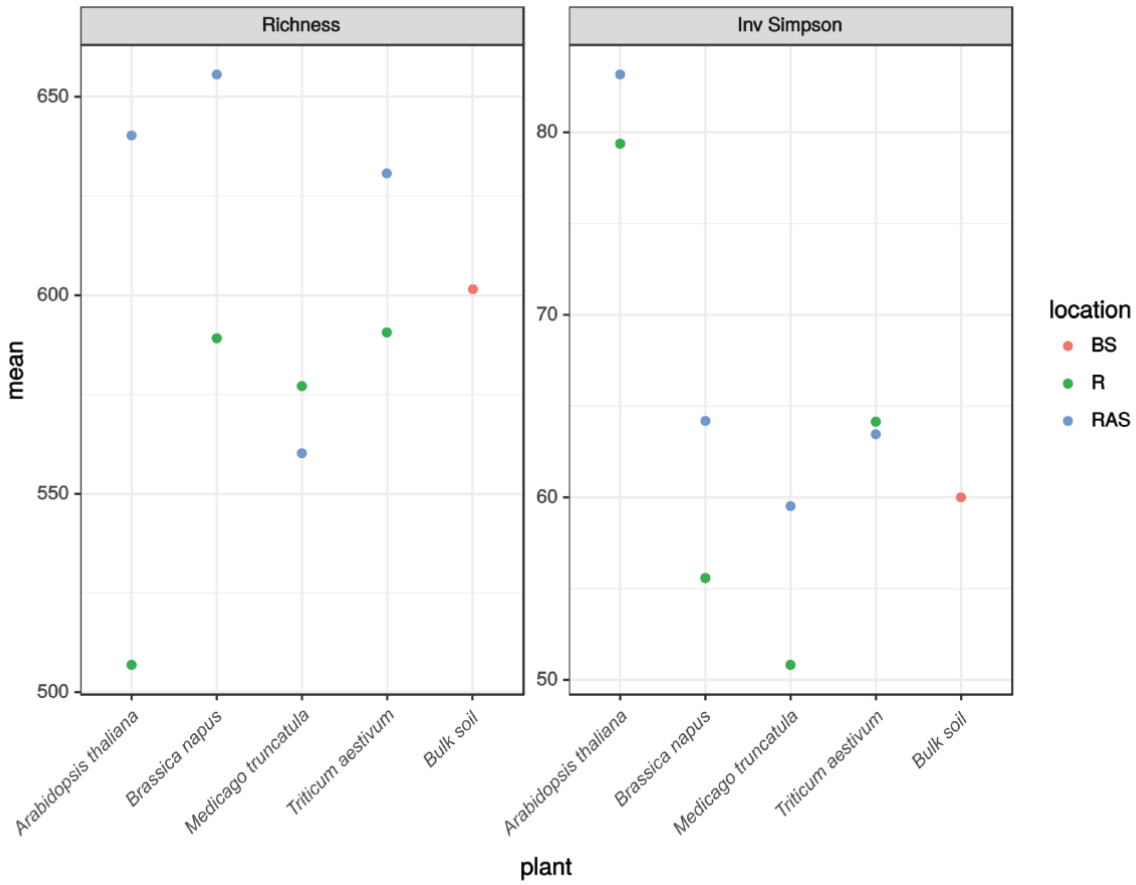

Figure S2

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