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Responses of soil microbiota and nematodes to application of organic and inorganic fertilizers in grassland columns

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Abstract

Enhancing the role of the soil microbiota in plant phosphorus (P) and sulfur (S) supply through application of organic fertilizer could reduce dependencies on non-sustainable synthetic fertilizers. To compare the effects of organic/inorganic fertilizers on the soil microbiota, soil columns with *Lolium perenne* (ryegrass) were setup in a greenhouse and amended with an inorganic fertilizer, cattle slurry (organic), or urea (P- and S-free control). Ryegrass rhizosphere of the slurry treatment had significantly higher abundances of bacterial-feeding nematodes, mycorrhizal colonization, cultivable heterotrophic bacteria, phosphonate- and sulfonate-utilizing bacteria, arylsulfatase activity, available P and *Variovorax asfA* gene copies compared to the inorganic and urea treatments. Phosphomonoesterase activities, and gene abundances involved in organic P and S transformations (*phoD*, *phoC*, *Burkholderia* & *Polaromonas asfA*) were similar in all treatments. Grass dry matter yield, shoot uptake of N, P and S were significantly higher in the inorganic treatment compared to the urea and slurry treatments. Community compositions differed significantly between the three fertilizer treatments and included the bacterial, alkaline phosphomonoesterase-producing bacterial, fungal, AM fungal, and nematode communities. *Bacteriodetes* were found in higher relative abundance in the organic treatment while *Acidobacteria* were more abundant in the urea and inorganic fertilizer treatments. These community shifts correlated significantly with grass dry matter yield, uptake of N, P and S, mycorrhizal colonization, enzyme activities, abundances of bacteria and bacterial-feeding nematodes. We concluded that organic fertilization promoted soil microbes and nematodes which have the potential to support sustainable plant growth, provided that the overall nutrient requirements are met.

Introduction

Phosphorus (P) and Sulfur (S) are essential macronutrients for all living organisms and required for plant nutrition (Kertesz and Mirleau 2004; Elser 2012). While mineable reserves of P are finite (Cordell et al. 2009), atmospheric deposition of S has declined substantially over the past 20-30 years (Fowler et al. 2005). Future supplies of both P and S for agricultural use remains less certain. Increasing demands in food production due to a growing world population further intensifies the demand for application of inorganic fertilizers in agriculture. In addition to issues around access, rising costs of fertilizers and environmental pollution associated with excessive use are of important concern. Alternative sources of plant nutrients such as manures are used to improve plant nutrient use efficiency and crop yield (Haynes and Williams 1993; Fageria and Baligar 2005; Chardon et al. 2007). In most cases, manure application leads to increased crop yields due to an increase in nutrient availability and improvement of soil structure (Matsi et al. 2003; Dordas et al. 2008).

In Ireland, the primary agricultural activity is grass-fed livestock for beef and/or milk production (Harris et al. 2011; O'Mara 2008). Animals are typically kept out on pasture from the months of March to October and are then kept indoors during the winter months. This is to protect the soil from trampling and compaction during the wetter winter months. This over-wintering produces a large quantity of manure (Harris et al. 2011). For instance, in 2003, 37 million tonnes of manure was produced by the Irish national cattle herd, 29.3 million tonnes of which was liquid slurry. Slurry is a heterogeneous mixture of animal feces, urine, small amounts of bedding and washings/rainwater and is typically stored in storage tanks on farms. The recycling of slurry back to the farm in the form of fertilizer is a common practice for the sustainability of the agricultural sector (Holden et al. 2004).

Organic and inorganic fertilizer application not only increase plant nutrient availability, but can also affect the activity and composition of the soil microbiota, thereby influencing nutrient turnover and plant growth (Marschner et al. 2003). Intensive agricultural production systems with high inputs of inorganic fertilizers substantially affect essential soil ecosystem services; nutrient cycling and functional biodiversity are mostly reduced (Hättenschwiler et al. 2005). Inorganic fertilizer applications have been reported to have negative effects on the abundance, activity and composition of soil microbiota and their ecosystem services (Ikoyi et al. 2018). The negative effect of inorganic fertilizer has been attributed to osmotic effects and/or changes in soil pH induced by the fertilizer application (Zhang et al. 2017). Specifically, significant decreases in nematode abundance were reported in a secondary tropical forest (Zhao et al. 2014) and in grassland columns (Ikoyi et al. 2018) after the application of phosphate fertilizer. Utilization of organic fertilizers may alter microbial

community composition as the C/N ratio influences decomposition rates (Martinez-Garcia et al 2018). Soil microbial communities play vital roles in the decomposition of organic inputs thereby affecting the biogeochemical cycling of nutrients and are vital in maintaining soil functioning (Martínez-García et al. 2018). A meta-analysis by Lori et al. (2017) has shown that application of organic fertilizers resulted in increased bacterial abundance and enzyme activities. They showed that land use, plant life cycle and climate were the factors responsible for the observed differences in microbial size and activity between soils receiving organic fertilizer inputs and those receiving inorganic inputs. Moreover, in comparison to the application of inorganic fertilizer, organic fertilizer has been shown to maintain a diverse soil microbiome (Cai et al. 2017), improve soil fungal interaction (Xue et al. 2018), and enhance soil microbial biomass and activity in a perennial grass sward (Neufeld et al. 2017). Therefore, organic fertilizers may have the potential to support plant growth without many of the negative effects of inorganic fertilizers on the soil microbiota and microfauna diversity and function.

There is a need to seek more sustainable methods to provide P and S for plant growth across the globe. Enhancing the role of the soil microbiota in nutrient supply could counteract present challenges around the finite nature of world's reserve of mineable rock phosphate and S deficiencies in soils due to reduced air pollution. The bacterial non-specific acid phosphomonoesterase (*phoC*) gene and alkaline phosphomonoesterase (*phoD*) gene are required for the hydrolysis of phosphomonoesters into orthophosphate for plant uptake (Fraser et al. 2015a; Lidbury et al. 2016; Fraser et al. 2017). Previous studies have shown that the activity of bacterial phosphate starvation (pho) regulon is induced under conditions of phosphate deficiency (Apel et al. 2007; Fraser et al. 2017). Moreover, the bacterial *asfA* gene, a member of the monoxygenase enzyme complex is required for the release of sulfate from aromatic sulfonates in soils (Kertesz and Mirleau 2004). The use of organic fertilizer as a source of P and S and its putative beneficial effect on the soil microbiota and nematodes involved in the cycling of P and S in soils has the potential to create a positive feedback towards sustainable plant growth. However, a lack of knowledge currently exists on the roles of arbuscular mycorrhiza (AM) fungi and nematodes (through their feeding activities) on P and S cycling in soils and the effects of organic and inorganic P and S fertilizer application. This is especially the case for grasslands, where these organisms could greatly influence the biological cycling of P and S alongside functional guilds of bacteria.

We hypothesized that the application of organic fertilizer stimulates the microbial release of P and S from soil and fertilizer, thereby contributing more to P and S availability in grasslands when compared to inorganic fertilizer use. This study aimed to provide an understanding of how the grassland

plant *Lolium perenne* is supported by the associated soil microbiota and nematodes in an organic and inorganic fertilization scenario that includes P and S. The objective was to assess the abundance, composition, and function of the soil bacteria, fungi, AM fungi and nematodes in organic and inorganic fertilization events and how this affects P and S cycling and uptake by *L. perenne*.

105 **Materials and methods**

106

107 **Experimental setup, column harvest, plant and soil analyses**

108 A P-limited (Irish soil P index 1) and moderate-S soil (equivalent to Wisconsin S soil availability index
109 of below 0.03 kg/m^3) sampled from Moorestown Cahir (County Tipperary, Ireland) was used to set up
110 soil columns in a greenhouse. Soils were sampled from 0-20 cm and 20-40 cm of the profile, sieved
111 through a 3.35 mm mesh to remove stones, mixed and repacked into the columns in layers of 0-20 and
112 20-40 cm as in the field. The columns were set up as in a similar way as a previous experiment (Ikoyi
113 et al. 2018). In brief, 16 cm x 40 cm pipes filled with soil were planted with ryegrass (*Lolium perenne*
114 variety Trend). Each treatment consisted of six replicates of: i) organic fertilizer (OF) as cattle slurry
115 (applied 5 cm below the column surface at an amount of 30 t ha^{-1} as published previously; Fox et al.
116 2017), ii) inorganic fertilizer (IF), full complement (20 kg ha^{-1} P, 20 kg ha^{-1} S, 125 kg ha^{-1} nitrogen
117 (N), 150 kg ha^{-1} potassium (K) and micronutrients) and iii) urea fertilizer as N only control (UC) (125
118 kg ha^{-1} N). The cattle slurry was obtained from pasture-grazed suckler cows (Taupe 2016) and was
119 applied during the column set up while the inorganic fertilizer and urea were applied in week seven
120 (concurrently to the first cut). The physicochemical properties including the nutrient concentrations of
121 the slurry are presented in Table S1. Rain water (200 mL) was added to the columns three times a
122 week. Rhizons (Rhizosphere Research Products, Wageningen, Netherlands) positioned in 10 cm height
123 intervals in the columns were used to collect soil solution samples weekly and analysed for anions
124 (phosphate, sulfate, nitrate, and other anions) using a Dionex ICS1100 ion chromatography system
125 with an AS23 column and the corresponding carbonate mobile phase according to the manufacturer's
126 guidelines (Dionex, Sunnyvale, CA). All treatments were managed for a period of 14 weeks.

127 After seven weeks of growth, grasses were cut back to 5 cm height and dried for dry matter
128 determination. At the end of 14 weeks of grass growth, the entire grass shoots were harvested and
129 dried for shoot dry matter determination. The columns were destructively harvested and rhizosphere
130 soil samples (soil retained on the roots after excess soil was shaken off roots) were collected for
131 determining acid and alkaline phosphomonoesterase activitivities, arylsulfatase activity, nematode
132 analysis and other analyses, while root samples were collected for mycorrhizal colonization analysis
133 (see below). For each replicate, six rhizosphere soil samples were collected and combined to form one
134 composite replicate sample.

For both cuts, dry shoot biomasses obtained by drying the grasses at 55 ^{o}C for 72 hours in a fan oven. The dried grass shoots (second cut) were analyzed for their elemental compositions at Lancrop Laboratories Ltd. (using atomic absorption spectroscopy, inductively coupled plasma spectrometry, titrations, and spectrophotometry, accredited to ISO/IEC 17025:2005).

Prior to the determination of plant-available P, soil samples were dried overnight at 40 °C and sieved (2 mm mesh). Morgan's extractable P (a proxy for plant-available P) was determined by extracting soil with sodium hydroxide-acetic acid solution (pH 4.8) in a 1:5 soil to solution ratio (Peech and English 1944). The extracts were filtered through Whatman No. 2 filter paper. P concentrations in the extracts were determined via colorimetry at 880 nm using a Camspec M500 UV-Visible Spectrophotometer (Camspec, UK) following the molybdate-ascorbic acid method (Murphy and Riley 1962). The soil pH was measured potentiometrically in a 1:2 soil to deionized water ratio (McCormack 2002).

Cultivation-dependent analysis of calcium-phosphate hydrolysing bacteria, P and S mobilizing bacteria, phosphomonoesterase and sulfatase activities, mycorrhizal root colonization and nematode abundance determination

The extraction of bacteria was done using the rhizosphere soil from the top 10 cm of the column following the method described previously by Fox et al. (2014). Briefly, 3 g of roots (after gently shaking off loosely attached soil) were added to 50 ml tubes containing 20 ml sterile saline solution and mixed via rotation (75 times per minute; Intelli-Mixer RM-2, Elmi Tech Ltd, Latvia) for 30 min at 4 °C. The resultant solution was used to make serial dilutions. The total heterotrophic bacteria (cultivated in Reasoner 2 media) and cultivable bacteria capable of utilizing P from phytate (phosphate-esters, MM2Phy) and phosphonoacetic acid (MM2PAA) as sole source of P, aromatic sulfonate (MM2TS, toluene sulfonate) as sole source of S, were quantified through a most probable number (MPN) approach in microtiter plates (Fox et al. 2014). Colony forming units (CFU) were established to determine the cultivable bacteria solubilizing P from tri-calcium phosphate (TCP) agar plates (Fox et al. 2014). The remaining rhizosphere suspension (without the roots) was centrifuged at 3645 x g for 15 min at 4 °C and the resultant pellet was stored at -20 °C for DNA extraction and further molecular studies.

Rhizosphere soil samples were analysed for potential acid phosphomonoesterase (ACP) and alkaline phosphomonoesterase (ALP) activities according to the method of Tabatabai and Bremner (1969). In brief, 1 g of soil was incubated with modified universal buffer at pH 6.5 (ACP) or 11.0

(ALP) using para-nitrophenyl phosphate (Sigma Aldrich, St. Louis, MO) as a substrate. Samples were filtered and diluted within the range of the standard curve. The intensity of the p-nitrophenol (yellow colour) released was measured using a spectrophotometer at a wavelength of 420 nm. Potential arylsulfatase activity was determined using the method of Tabatabai and Bremner (1970a). Briefly, 1 g of soil was incubated for 1 h at 37 °C with acetate buffer (pH 5.8) using para-nitrophenyl sulfate (Sigma Aldrich, St. Louis, MO) as a substrate. The p-nitrophenol colour intensity of the filtered samples was measured with a spectrophotometer at 400 nm.

The percentage of grass roots colonized by arbuscular mycorrhizal (AM) fungi was determined following the grid line intersect technique (McGonigle et al. 1990) as modified by Ikoyi et al. (2018). The stained root segments were assessed under the microscope for the presence of AM structures (arbuscules, vesicles and hyphae), one field of view at a time. Three replicates of 100 intersections were used to calculate the root AM fungal colonization for each column.

Nematodes were extracted from 100 g fresh soil using the modified Baermann method (Whitehead and Hemming 1965) previously described by Ikoyi et al. (2018). The extracted nematodes were counted and divided into two portions. One portion was used for microscopy where nematodes were identified to the genus or family level based on morphological features and identification keys using a light microscope (Andrássy 1984; Siddiqi 1986; Bongers 1988; Jairajpuri and Ahmad 1992). The other portion was used for nematode DNA extraction (see below) and further molecular analyses (PCR-DGGE and sequencing). The identified nematodes were assigned to feeding groups and abundances were calculated based on total nematode counts.

DNA extraction, community fingerprinting and quantitative PCR

The PowerSoil DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA) was used to extract DNA from 0.25 g of the frozen soil pellets following the manufacturer's recommendations. The extracted DNA was quantified using a Qubit dsDNA HS Assay kit (Life Technologies, Carlsbad, CA) and a Qubit Fluorometer (Life Technologies). PCR amplifications of the bacterial (16S rRNA), fungal (ITS) and AM fungal (18S rRNA) gene fragments for denaturing gradient gel electrophoresis (DGGE) were performed as described previously (Table S2; Schmalenberger and Noll 2014; Ikoyi et al. 2018).

DNA fingerprinting of alkaline phosphomonoesterase-harbouring bacteria communities was performed using the primers ALPS-F730 and ALPS-R1101 targeting the bacterial *phoD* gene (Sakurai et al. 2008). A GC clamp was attached to the reverse primer and PCR was performed following the

198 methods described by Fraser et al. (2015b). DGGE was run using a urea-formamide gradient of 40–
199 70 %.

200 For fingerprinting of the nematode community composition, the second portion of the extracted
201 nematodes (see 2.2 above) was first concentrated on cellulose nitrate membrane filters (Whatman
202 Limited, UK; 25 mm diameter, pore size = 0.45 µm) and then nematode DNA was extracted from the
203 filters using the PowerSoil DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA) following the
204 manufacturer's instruction. PCR amplification of the nematode DNA was carried out using the primers
205 SSU18A and SSU9R-GC targeting the small subunit (SSU) of the 18S rRNA gene (Table S2; Okada
206 and Oba 2008). Each 25 µl reaction contained 1 x buffer (2mM $MgCl_2$), 0.2 mM dNTP mix, 0.4 mmol
207 of each primer, 0.5 U of DreamTaq polymerase (Fisher Scientific, Waltham, MA) and 1 µl of template
208 DNA. The PCR cycling conditions were as follows: initial denaturation at 95 °C (3 min), 35 cycles of
209 95 °C denaturation (30 s), annealing at 52 °C (30 s), 72 °C extension (1 min), and a final extension at
210 72 °C (10 min). DGGE was run as before using a urea-formamide gradient of 25-55 %.

211 All DGGE images were analyzed using the phoretix 1D software (Totallab, Newcastle, UK).
212 Canonical correspondence analyses (CCA) biplots were generated from the binary gel image matrices.
213 Permutation tests (Monte Carlo, 9999 repeats) were conducted using CANOCO (Microcomputer
214 Power Inc., Ithaca, NY). This allowed the identification of environmental properties affecting the
215 community compositions and significant differences between the samples (Noll and Wellinger 2008).

216 Absolute quantification of bacterial *phoD* genes was carried out using the extracted DNA from
217 the rhizosphere soil by amplifying with primers ALPS-F730 and ALPS-R1101 (Table S2; Sakurai et
218 al. 2008) in a LightCycler ® 96 Real-time PCR System (Roche, Basel, Switzerland). The PCR reactions
219 were prepared with 5 µl of KAPA SYBR FAST qPCR Universal Master Mix (Kapa Biosystems,
220 Boston, MA), 3 pmol of each primer, and 1 µl template in a 10 µl reaction. The cycling conditions
221 were as follows: enzyme activation at 95 °C for 3 min, 40 cycles of denaturation at 95 °C for 3 s,
222 annealing at 65-57 °C for 20 s (touchdown, -1 °C per cycle) and an extension at 72 °C for 20 s. Samples,
223 standards (10^2-10^4 molecules per reaction) and non-template controls were run in triplicates on 96-well
224 microtiter plates. To check the specificity of the reaction, a melt curve analysis was conducted
225 immediately following the run. Absolute quantification of bacterial *phoC* genes was done similarly to
226 *phoD*, using primers phoc_A_F1 and phoc_A_R1 (Table S2; Fraser et al. 2017) instead. Absolute
227 quantification of *Variovorax asfA* genes was carried out similarly to *phoD* from above, but using
228 primers asfA_Varx_F1 and asfA_Varx_R1 (Table S2; Schmalenberger et al. 2008) and cycling
229 reaction conditions used were: enzyme activation at 95 °C for 5 min, 40 cycles of denaturation at 95

°C for 10 s, annealing at 60 °C for 20 s and an extension at 72 °C for 10 s. Quantification of *Burkholderia* and *Polaromonas asfA* genes were performed as for *Variovorax asfA*, using the pimer pairs: asfA_Brk_F1 and asfA_Brk_R1, and asfA_Pol2_F1 and asfA_Pol2_R1 (Table S2; Ikoyi et al. 2019), respectively.

Amplicon sequencing and sequence analyses

Sequencing of the bacterial community was carried out at the Novogene Bioinformatics Technology Co., Ltd. Briefly, the V3-V4 region of the bacterial 16S rRNA gene was amplified using the 341F/806R (Takahashi et al. 2014) primer set (Table S2). The phusion high-fidelity PCR Mastermix (New England Biolabs, Ipswich, MA) was used to perform the PCR reactions and a Gel Extraction Kit (Qiagen, Dusseldorf, Germany) was used to purify the PCR products. NEBNext® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) was used for generating sequencing libraries following the manufacturer's instructions. Qubit Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system were used to assess library quality. The libraries were then sequenced on an Illumina HiSeq 2500 platform and 250 bp paired-end reads were generated. Bioinformatic analyses were performed using QIIME 2 2019.7 (Bolyen et al. 2019). Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin followed by denoising with DADA2 (Callahan et al. 2016) (via q2-dada2) to produce amplicon sequence variants (ASVs). All ASVs were aligned with mafft (Katoh et al. 2002; via q2-alignment) and used to construct a phylogeny with fasttree2 (Price et al. 2010; via q2-phylogeny). Alpha-diversity metrics (observed ASVs and Faith's Phylogenetic Diversity; Faith 1992), beta diversity metrics (weighted UniFrac (Lozupone et al. 2007), unweighted UniFrac (Lozupone and Knight 2005), Jaccard distance, and Bray-Curtis dissimilarity), and Principle Coordinate Analysis (PCoA) were estimated using q2-diversity after samples were rarefied. Silva version 132 99% reference data set for 16S rRNA (Quast et al. 2013) were obtained and trained for assigning taxonomy using the q2-feature-classifier (Bokulich et al. 2018). The first step in training the reference data set was to trim the sequences to the length of the reads using the PCR primers used to amplify the amplicons. A Naïve Bayes classifier was trained using the trimmed sequences reference sequences and their reference taxonomies. Non-metric multidimensional scaling (NMDS) analysis was performed based on the obtained ASVs with the R software (Version 2.15.3) using the vegan and mvabund packages. In addition, taxa bar plots were created to display the percentage relative abundance distribution of dominant taxa among the treatments. Nucleic acid sequences were deposited in the Nucleotide Archive (Project PRJEB33483; ERS3578501 - ERS3578518).

For the nematode community, amplicon libraries were generated by amplifying the 18S rRNA gene fragment of the nematode DNA using the primer set developed by Porazinska et al. (2009) but with illumina adapters (Nex_NF1 and Nex_18Sr2b). PCR amplifications were performed in triplicates. Each 25 µl reaction contained 1 µl of nematode DNA as template, 5 µl of 5x KAPA HiFi Fidelity Buffer, 0.5 µl of 10 mM dNTP, 0.2 µl of each primer (forward and reverse) and 0.1 µl of KAPA HiFi HotStart DNA polymerase (Kapa Biosystems, Boston, MA). The PCR cycling condition included enzyme activation at 95 °C for 3 min, 35 cycles of denaturation at 98 °C for 20 s, annealing at 62 °C for 15 s, extension at 72 °C for 15 s. A final extension was performed at 72 °C for 1 min. The triplicate PCR products were pooled and purified using GenElute PCR Clean-Up Kit (Sigma-Aldrich, St. Louis, MO). A second PCR was conducted, to add indexes (7 bp dual indexes) and Illumina flow cell adaptors, using 10 µl reactions containing: 0.5 µM of each index primer (indexes were based on Kircher et al. 2012; Gansauge and Meyer 2013), 5 µl 2X KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Boston, MA), and 2 µl of previous PCR product diluted 1:4 with 10 mM Tris. The thermal cycling condition consisted of an enzyme activation step at 95 °C for 3 min; 10 cycles including denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. A final extension was carried out at 72 °C for 5 min. The resulting PCR products were cleaned using 0.9 X by volume AMPure XP beads (Beckman Coulter) following the manufacturer's instructions. The concentration of each cleaned PCR product was measured using Nanodrop 2000 (Thermo Fisher Scientific), and PCR products were normalized to 15 nM using 10 mM Tris pH 8.5. The final pool was created by combining an equal volume from each normalized sample. The concentration of the final pool was assessed by qPCR using KAPA Illumina Library Quantification (Kapa Biosystems, Boston, MA) following the manufacturer's instructions. Illumina paired-end sequencing was performed using a 500 cycle Illumina MiSeq V2 Kit (Illumina) on an Illumina MiSeq sequencer at CIBIO-InBio (Vairão Campus, Portugal).

Bioinformatic analysis of the nematode sequences was performed in a similar way as the bacterial 16S rRNA. The only exception was that the Silva version 132 99% reference database for 18S rRNA (Quast et al. 2013) was trained and used for the taxonomy assignment and the trimming of the sequences to read lengths was with the nematode primers used for the amplicon sequencing . Non-metric multidimensional scaling (NMDS) analysis was performed based on the obtained nematode ASVs with the R software (Version 2.15.3) using the vegan and mvabund packages. Nucleic acid sequences were deposited in the Nucleotide Archive (Project PRJEB33483; ERS3578525 - ERS3578542).

293

294 **Statistical analyses**

295 Prior to statistical analyses, data on grass elemental composition were converted to unit per column
296 basis in order to capture the total dry matter yield and mass balances. The grass dry matter yield and
297 elemental composition, MPN, CFU, acid and alkaline phosphomonoesterase activities, arylsulfatase
298 activity, percentage root mycorrhizal colonization, and abundance of nematode feeding groups were
299 analyzed using a one-way ANOVA in R software (Version 2.15.3). Statistical significance was tested
300 at $P < 0.05$. Shapiro-Wilk's and Levene's tests were used to check data for normality and homogeneity
301 of variance respectively. Tukey's HSD post-hoc test was applied for pairwise comparisons to assess
302 any significant differences ($P < 0.05$) between treatment means, when normality and homogeneity of
303 variance was confirmed. Data violating the model assumptions were logarithmically transformed,
304 analysed by ANOVA and the back transformed values to the original scale were reported. Where
305 homogeneity of variance was not achieved, the Games-Howell test was used and where both
306 assumptions of ANOVA were not satisfied, the Kruskal-Wallis test was performed instead. In addition,
307 Spearman's rank correlation analyses were carried out to identify potential relationships between soil
308 nutrient (N, P, S) contents and other measured variables.

309

Results

Soil chemical properties, grass dry matter yield and elemental composition, soil pH and Morgan's P

The weekly measurements of phosphate in soil solution at four different soil depths for the duration of 14 weeks showed a higher amount of phosphate in the organic fertilizer treatment (OF) for most of the weeks in the top 20 cm of the column (Figure 1a). The phosphate concentration was similar in the inorganic fertilizer (IF) and urea-only control (UC) treatments except in weeks 7 and 8 when it was significantly higher in the inorganic fertilizer treatment (fertilization event). Sulfate concentration had a similar trend (Figure 1b). A significant increase in sulfate concentration was observed in IF in week 7 and 8 due to the fertilizer application. The sulfate concentration was significantly higher in the OF and IF treatments when compared to UC from week 7 until the end of the experiment. The nitrate concentration was similar in IF and UC but higher compared to the OF treatment from week 4 to the end of the experiment (Figure 1c). The total grass dry matter yield (sum of both cuts) was significantly higher in the IF and UC treatments than in the OF treatment ($P<0.05$, Table 1). The analyses of the elemental composition of the grass dry matter revealed that the IF treatment had a significantly higher ($P<0.05$) uptake of P, S, N, K, Ca, Zn, B and Mg (Table 1) per column. The OF treatment had a significantly higher pH and available P concentration (as extracted by Morgan's solution) compared to IF and UC treatments (Table 2).

Effect of treatments on abundance of cultivable bacteria, enzyme activities, mycorrhizal root colonization and abundance of nematodes

The abundances of cultivable heterotrophic, phosphonate-utilizing and sulfonate-utilizing bacteria were significantly higher ($P<0.05$) in the OF treatment compared to the IF and UC treatments (Table 2). Sulfonate and phosphonate utilizing bacteria were about 3 and 5 times more abundant in OF than in IF, respectively. The treatments had no significant effect ($P>0.05$) on the abundances of phytate-utilizing and calcium phosphate-utilizing bacteria which ranged from 2.25-5.99 x 10^7 MPN g^{-1} and 7.00 x 10^5-1.07 x 10^6 CFU g^{-1}, respectively (Table 2). The use of TCP to isolate Ca-P-solubilizing bacteria did not assess the bacterial ability to solubilize other forms of inorganically bound P and did only identify bacteria, capable of hydrolyzing the Ca-P bond. The potential alkaline phosphomonoesterase (ALP) and acid phosphomonoesterase (ACP) activities were statistically similar

in the OF and IF treatments but ALP was significantly higher in the OF than in the UC treatment (Table 2). For the arylsulfatase activity, the OF treatment had a significantly higher activity by about 20 % when compared to the IF and UC treatments (Table 2). *Lolium perenne* roots of the OF and UC treatments had significantly higher percentage of roots with mycorrhizal arbuscules, hyphae and vesicles ($P<0.05$; Table 2) than the IF treatment. There were about 27 to 34 % more arbuscules, 15 to 22 % more vesicles, and 30 to 35 % more AM hyphae in the UC and OF treatments than in the IF treatment. The total nematode abundance was significantly higher ($P<0.05$) in the OF treatment when compared to the IF and UC treatments. The abundance of bacterial-feeding nematodes was significantly higher in the OF treatment than in the UC treatment and lowest in the IF treatment ($P<0.05$). Bacterial feeders were about 40 and 71 % more abundant in the OF treatment than in the UC and IF treatments, respectively (Table 2). The higher total nematode abundance and abundance of bacterial-feeding nematodes in the OF treatment was due to higher prevalence of bacterial-feeding nematodes belonging to the Cephalobidae and Rhabditidae families (Table S3).

Microbial and nematode community fingerprints

The DGGE fingerprint of bacterial 16S rRNA gene fragments with the various replicates is shown in Figure S1. The resulting biplot (CCA) of the bacterial community composition showed that the UC and IF treatments overlapped on the first axis (Figure 2a). The OF treatment however, was clearly separated from the IF treatment on both axes, and separated from the UC on the first axis (Figure 2a). Permutation tests of the bacterial community DGGE profile confirmed a significant separation between all treatments ($P<0.05$). CCA and permutation analysis of the bacterial community profiles revealed a highly significant correlation of total shoot dry matter (TDWT), pH, shoot N, P and S contents, potential alkaline phosphomonoesterase (ALP), acid phosphomonoesterase (ACP) and arylsulfatase (ASULF) activities, soil Morgan's P concentration (AvP), total nematode abundance (TNEM), abundance of bacterial-feeding nematodes (BF), abundance of cultivable calcium-phosphate utilizing bacteria (TCP), and *Variovorax asfA* gene copies (Var) with the bacterial community composition.

For the community composition of the alkaline phosphomonoesterase-harbouring bacteria, the resulting CCA biplot showed that while there was an overlap between all treatments on the first axis, IF and OF treatments were clearly separated on the second axis (Figure 2b). In contrast, UC partially overlapped with both fertilizer treatments (IF and OF) on the second axis. Permutation tests confirmed a significant separation between the UC/OF treatments and IF treatment ($P<0.05$). CCA and

372 permutation analysis of the alkaline phosphomonoesterase-harbouring bacterial community profiles
373 revealed a highly significant correlation of the community composition with total shoot dry matter
374 (TDWT), shoot P content, potential ALP activity, pH, soil Morgan's P concentration (AvP),
375 abundances of cultivable total heterotrophic, phytate-utilizing (PHY), phosphonate-utilizing (PAA)
376 and calcium-phosphate-utilizing bacteria (TCP), and abundance of bacterial-feeding nematodes (BF).

377 The DGGE fingerprint of the fungal ITS with the various replicates is shown in Figure S2. The
378 CCA biplot of the community composition of the higher fungi revealed a clear separation between the
379 UC/IF and OF treatments on the first axis (Figure 2c). On the second axis however, a separation
380 between the IF and UC treatments was clearly visible. All three separations were confirmed to be
381 significant ($P<0.05$) by permutation tests. These separations were significantly ($P<0.05$) associated
382 with total shoot dry matter (TDWT), shoot N, P and S contents, pH, soil Morgan's P concentration
383 (AvP), percentage of root colonized by mycorrhizal arbuscules (AC), vesicles (VC) and hyphae.

384 For the community composition of the AM fungi, the CCA biplot and permutation tests
385 revealed a highly significant separation ($P<0.01$) between the IF treatment, UC and OF treatments
386 (Figure 2d). IF, UC and OF were clearly separated on the first axis, but not on the second axis.
387 Moreover, similar to the fungal community composition, the separation was significantly ($P<0.05$)
388 correlated with total shoot dry matter (TDWT), shoot N, P and S contents, pH and percentage of roots
389 colonized by mycorrhizal arbuscules (AC), vesicles (VC) and hyphae.

390 The CCA biplot of the nematode community composition showed that there was a clear
391 separation between UC and OF treatment on the first axis, and UC/OF versus IF treatment on the
392 second axis (Figure 2e). Permutation tests confirmed a highly significant separation between all
393 treatments ($P<0.01$). CCA and permutation analysis of the nematode community profiles revealed a
394 highly significant correlation of the nematode community composition with total shoot dry matter
395 (TDWT), shoot N, P and S contents, pH, soil Morgan's P concentration (AvP), percentage of roots
396 colonized by mycorrhizal arbuscules (AC), vesicles (VC) and hyphae, and abundances of bacterial-
397 feeding (BF) fungal-feeding (FF) nematodes.

398

399 **Gene copies of *phoD* and *phoC*, and abundance of *Variovorax*, *Burkholderia* and *Polaromonas***
400 ***asfA* genes**

401 In general, the *phoD* copy numbers were higher than *phoC* copies in all treatments (Table 2). The *phoD*
402 and *phoC* gene copy numbers did not differ significantly ($P>0.05$) between the treatments. Numbers

403 ranged from 3.28 to 3.33 x 10^5 and 2.66 to 2.98 x 10^5 copies per gram of dry soil for the *phoD* and
404 *phoC* genes, respectively. The OF treatment had significantly higher copies of *Variovorax asfA* genes
405 (about 2.5 to 3 times higher) than the UC and IF treatments (Table 2). Gene copy numbers of the
406 *Burkholderia* (1.2-2.0 10^6 g^{-1}) and *Polaromonas* (1.2-2.1 10^5 g^{-1}) *asfA* were statistically similar in all
407 treatments (Table 2).

408

409 **Bacterial and nematode diversities and community compositions based on next generation**
410 **(amplicon) sequencing**

411 Sequences of the 16S rRNA gene (amplicons) allowed the estimation of alpha and beta diversities of
412 the treatments using QIIME 2. The alpha diversity in the experiment ranged from an average of 9.68
413 to 9.84 (Shannon) or 1621 to 1769 (observed species), respectively (Table S3). The alpha diversity
414 indices were not statistically different among the various treatments. The rarefaction curve for the
415 bacterial 16S rRNA sequences is shown in Figure S3. NMDS analysis revealed a clear separation
416 between the OF treatment from the UC and IF treatments (beta-diversity; Figure 3). Permutational
417 analysis of variance (PERMANOVA) indicated a significant separation of the OF from the IF
418 (P=0.002) and UC (P=0.005) treatments. In addition, analysis of similarities (ANOSIM) confirmed
419 this separation of the OF versus IF (R = 0.32, P = 0.001) and UC (R = 0.28, P =0.008) treatments.
420 Moreover, linear discriminant analyses (LDA) revealed that bacteria belonging to *Bacteroidetes*
421 phylum were more abundant in the OF treatment, while in the UC and IF treatments, members of the
422 *Acidobacteria* phylum were more abundant (Figure S4). Furthermore, the OF treatment had a
423 significantly higher (P<0.05) abundance of genera belonging to *Acidibacter*, *Burkholderia,*
424 *Allorhizobium, Terrimonas, Dyella, Ferruginibacter*, *Chrysolinea, Devosia*, *Pelomonas, Hirschia,*
425 *Ohtaekwangia, Actinocorallia, Altererythrobacter, Asticcacaulis* and *Mucilaginibacter* than in the IF
426 treatment (Figure 3b).

427 Analysis of the sequences of the SSU of the 18S rRNA gene sequence (nematode amplicons)
428 showed that 58 % of the reads of the detected taxa were nematodes (Figure S5). Analysis of the
429 nematode faunal assemblage showed that the OF treatment was dominated by bacterial-feeding
430 nematodes (particularly, members of the Rhabditidae family) while plant- and fungal-feeding
431 nematodes were more abundant in the IF treatment, which was a similar pattern as observed with the
432 nematode analysis based on morphology (Figure S6). The rarefaction curves for the nematode 18S
433 rRNA sequences are shown in Figure S7. NMDS analysis of the ASVs revealed a significant separation

between the OF treatment from the IF treatments (Figure 4), while UC partially overlapped with OF and IF. PERMANOVA showed a significant separation of the OF from the IF (P=0.035) and UC (P=0.029) treatments. Moreover, ANOSIM results confirmed the significant separation of the OF from the IF treatment (R=0.36, P=0.02).

Relationship between soil nutrient (N, P and S) contents and other measured variables

Spearman's rank correlation analyses (Figure S8) showed that nitrate content of soil (soilN) significantly (P<0.05) correlated positively with the shoot dry matter but negatively with abundance of bacterial-feeding nematodes, arbuscular mycorrhizal hyphal colonization, arysulfatase activity, soil pH and soil P. Soil P content significantly (P<0.05) correlated negatively with *Burkholderia asfA* gene copies, shoot dry matter, shoot K and shoot N but positively with mycorhizal vesicular colonization, abundance of bacterial-feeding nematodes, available P (Morgan's P), *Variovorax asfA* gene copies, total cultivable heterotrophic bacteria (R2A), soil S and soil pH. Soil S content significantly (P<0.05) correlated positively with shoot S, available P (Morgan's P), *Variovorax asfA* gene copies and abundance of total cultivable heterotrophic bacteria. Moreover, arylsulfatase activity significantly (P<0.05) correlated positively with abundance of bacterial-feeding nematodes and mycorrhizal colonization but significantly (P<0.05) correlated negatively with soil N, shoot N and shoot dry matter.

Discussion

In this study, the phosphate concentration was significantly higher in the soil solution of organic fertilizer (OF) than in the inorganic fertilizer (IF) and the urea-only control (UC) treatments, with the exception for week 7-8 immediately after the inorganic fertilizer and urea application. Moreover, the highest amount of Morgan's P (a proxy for plant-available P) was obtained in the OF treatment. Inorganic forms of P from the cattle slurry may have contributed to the increase in Morgan's P. However, the activity of soil microbes in the soil breaking down organic P forms in the organic fertilizer into plant available form for plant uptake may have also been a contributor. Indeed, the significantly higher abundance of cultivable heterotrophic bacteria and bacteria capable of utilizing phosphonoacetic acid (carbon bonded P), higher mycorrhizal colonization and abundance of bacterial-feeding nematodes in the OF treatment supports this hypothesis. Higher levels of available P due to organic fertilizer use have been reported before. For instance, Steiner et al. (2007) found that soils had a significantly higher level of available P after chicken manure application than after inorganic fertilizer application.

The sulfate concentration in soil solution was similar or significantly higher in the OF when compared to the IF treatment in weeks 4-6 and 9-14. The breakdown of organo-S forms by soil microbes and the subsequent release as sulfate into the soil solution may have contributed to this. Indeed, the significantly higher abundance of aromatic sulfonate-utilizing bacteria in the OF treatment supports this claim. The breakdown of sulfonates to release S into soil solution has been linked previously to the activity of microbes (Kertesz and Mirleau 2004). While the present study has not quantified the ratio of organic and inorganic S in the applied slurry, previous reports have highlighted the high content of sulfonates in animal feces such as the reports by Williams and Haynes (1993) for sheep dung that contained 80 % of the S as sulfonates. In contrast to potential ALP and ACP activities that remained largely unchanged, significantly higher arylsulfatase activity as well as higher abundances of aromatic sulfonate utilizing bacteria were recorded in the OF compared to the IF and UC treatments. High arylsulfatase activity has been attributed to increases in soil organic matter content (Tabatabai and Bremner 1970b). Incorporation of manure and other organic material into the soil has been shown to increase soil organic matter content (Pascual et al. 2000; Marschner et al. 2003). It is likely that production of this enzyme contributed to the release of sulfate from hydrolysis of sulfate-esters into the soil solution. As a consequence, similar or higher levels of sulfate were found in the OF than in the IF treatment with the exception of the two weeks post IF application.

AM fungal symbiosis has been reported to improve the nutritional status of the host plant (Smith and Smith 2012), particularly under conditions of low nutrient availability. A significantly higher AM fungi colonization of ryegrass roots in the OF and UC treatment when compared to the IF treatment was observed in this study. Mycorrhizal symbiosis has been reported to be suppressed by phosphate addition (Bentivenga and Hetrick 1992; Baum and Makeschin 2000; Ikoyi et al. 2018). Similar to the phosphate effect, AM fungal acquisition of sulfate is particularly important in soils that are poor in S levels (Sieh et al., 2013). Consequently, a reduction in AM fungal colonization by the IF treatment will negatively impact ryegrass nutrient uptake that includes P and S.

Similar to the AM fungi response, the total nematode abundance and in particular the abundance of bacterial-feeding nematodes was significantly reduced in the IF treatment. It has been reported previously that soil nematodes are sensitive to salt toxicity and negative effects of high phosphate application on soil nematodes (attributed to salt toxicity) has been described (Zhao et al. 2014; Ikoyi et al. 2018). It is likely that the application of inorganic fertilizer resulted in increased salt concentrations, hence the negative response from the bacterial-feeding nematodes. In contrast, application of organic manure to grassland has been widely reported to increase the abundance of nematodes, particularly due to increased substrate availability and altered soil conditions (Bardgett and Griffiths 1997; Griffiths et al. 1998; Neher 1999; Forge et al. 2005). Microbial feeding nematodes are regarded as the most important grazers of microbes (bacteria and fungi) in terrestrial ecosystems (Ingham et al. 1985; 1986). Soil nematodes (particularly bacterial- and fungal-feeding nematodes) play significant roles in biological cycling of nutrients due to their feeding activities. For instance, increased nitrate and P availability have been linked to nematode grazing on rhizosphere bacteria (Irshad et al. 2011; Gebremikael et al. 2016). The reduction in abundance of bacterial-feeding nematodes in the IF treatment will limit their contribution to P and S cycling.

Unexpectedly, a significantly lower grass growth was obtained in the OF treatment compared to IF and UC treatments. This is in contrast to a previous study where a higher biomass yield of wheat was obtained in organic fertilizer treatment (pig slurry) compared to inorganic fertilizer treatment (Abubaker et al. 2012). However, their application amount (58.3 t ha^{-1}) exceeded the amount applied in normal agricultural practices. N limitation could be a possible explanation for the low grass productivity in the OF treatment as most of the nitrate in the soil solution was lost from week four of the experiment. The OF application increased soil nitrate concentration only in the first four weeks when the ryegrass plants were not fully developed. This was not sufficient enough to sustain the grass productivity through the 14-week growth period. Plants and soil microbes require a well-balanced

supply of N, P and S for their growth and development (Cleveland and Liptzin 2007; Hartman and Richardson 2013). As a major component of chlorophyll, N in plants is important in photosynthesis and plant growth is limited under conditions of N limitation (Vance 2001). In addition, even though microbial mineralization of organic N in the organic fertilizer should have increased N availability, the high C/N ratio of the OF (28.4) probably stimulated microbial N assimilation rather than mineralization. When organic amendments with C/N ratios higher than 20 are added to soils, net assimilation often dominates (Myrold 1999; Calderon et al. 2004). Manure application at C/N ratios below 20 supports net mineralization and this has been reported to increase the dry matter yield of tall fescue (Shi et al. 2018). Thus, N limitation was a major hindrance to plant growth in the OF treatment. Due to the higher IF grass yield, the total uptake of P, S, N and K by the ryegrass was significantly higher in the IF treatment compared to the OF treatment, despite the higher concentrations of phosphate and sulfate in soil solution in the OF treatment. The present study used slurry and inorganic fertilizer amounts that reflect agricultural practice for grasslands in Ireland. However, the total N input through OF use was lower than in comparison for IF and UC which may have contributed to the N limitation in the OF treatment. While repeated application of OF may alleviate some of this limitation over an extended period of time, future sustainable fertilization strategies may consider blending of OF with urea or other sustainable sources of N that are less likely to disrupt soil biota functions. Another potential issue around the use of animal manures as soil amendments is that their application is also a potential route for the accumulation of heavy metals, antibiotics and pathogens in the soil (Chee-Sanford et al. 2009; Hejna at al. 2018). However, this is a more general problem of intensive farming practices where animals are fed to obtain short-term gains over long-term sustainability and animal welfare.

Molecular methods have been used in the present study to obtain further insights into the microbial and nematode population. This included the community analysis of bacteria, fungi, nematodes as well as the abundance of functional key genes in P and S cycling and mineralization. Although copies of *phoD* and *phoC* genes appeared to be slightly higher in OF than the other two treatments, this was not statistically significant. Furthermore, there was no significant correlation between potential ALP and ACP activities and *phoD* and *phoC* gene abundance which is consistent with previous reports (Fraser et al. 2015a; Chen et al. 2018). Bacteria may have more than one copy of the target gene per genome as demonstrated for *phoD* where up to 9 copies per genome were reported by Ragot et al. (2015). Therefore, the gene copies may not accurately reflect the actual bacterial abundance after all (Fraser et al. 2017). Moreover, the production of ALP and ACP is linked to multiple

548 genes in bacteria. Bacterial *phoA*, *phoD* and *phoX* genes have been associated with ALP production
549 (Apel et al. 2007; Lidbury et al. 2016), while nonspecific acid phosphomonoesterases have been
550 grouped into class A-C (Gandhi and Chandra 2012). The *phoC* gene belonging to class A was the target
551 in this study. Wei and colleagues (2019) found that in paddy soils without fertilization, rare *phoD* taxa
552 become more prominent. To fully understand the contribution of microbial phosphomonoesterases to
553 P mineralization in these columns, there is need for further investigation into the sequences of *phoD*
554 and *phoC* metagenome and metatranscriptome. Microbial mineralization of phosphonate may be
555 playing a role in the phosphate availability in the OF treatment as there was a significant increase in
556 the abundance of bacteria capable of utilizing phosphonoacetic acid. Efforts in the present study to use
557 primers targeting *phnJ* to quantify the abundance of this phosphonatase gene in a qPCR assay failed.
558 Future studies are needed to develop protocols to quantify phosphonatase genes accurately.

559 The copies of *Variovorax asfA* gene obtained via qPCR were significantly higher in the OF
560 than the IF and UC treatments. *Variovorax* has been reported previously as a functional specialist for
561 sulfonate cycling in wheat rhizosphere (Schmalenberger et al. 2008). As animal manures have been
562 reported in the past to be sulfonate-rich (William and Haynes 1993), some bacteria with the ability to
563 utilize aromatic sulfonates as a source of S (evident through the presence of *asfA*) may play an
564 important role in the desulfonation process and ultimately in plant S supply. While this may be the case
565 for *Variovorax* in the present study, *Burkholderia* and *Polaromonas asfA* gene copies were statistically
566 similar in all three treatments. Total copy numbers of bacterial *asfA* as well as their expression may
567 shed further light onto the potential for sulfonate desulfurization. Until now, the difficulty to quantify
568 *asfA* universally has hampered the development of a suitable qPCR protocol.

569 The bacterial (16S rRNA), bacterial phosphomonoesterase (ALP), fungal (ITS), AM fungal
570 (18S rRNA) and nematode (18S rRNA) community compositions as visualized via DNA fingerprinting
571 (PCR-DGGE) were affected by the treatments in the present study. Community fingerprint analysis is
572 limited to the comparison of dominant clades in the overall communities. However, general trends can
573 be reliably identified, which can be confirmed in far greater detail via high throughput sequencing. In
574 the present study, this was carried out for the bacterial and nematode communities, but not for the
575 fungal communities. In most cases, the IF treatment clustered closely together and correlated with total
576 shoot dry weight, shoot uptake of N, P and S. The OF and UC treatments clustered separately from the
577 IF treatment, and significant correlations with enzyme activities, mycorrhizal colonization, nematode
578 abundance and pH were found alongside the abundance of bacteria involved in the cycling of P and S.
579 These findings are in accord with previous studies that identified significant correlations between shifts

580 in the bacterial phosphomonoesterase communities, alkaline phosphomonoesterase activity (as soil
581 enzymatic test) and fertilizer application have been reported (Sakurai et al. 2008; Chhabra et al. 2013).
582 In the present study, the abundance of bacterial-feeding nematodes correlated significantly with shifts
583 in the bacterial, ALP-harboring bacterial and nematode community compositions. Recently, nematode
584 grazing was reported to promote soil bacterial community dynamics with a positive relationship
585 between dominant bacterial feeders and ALP-producing bacteria (Jiang et al. 2017). In addition, shifts
586 in the nematode community composition identified in the present study correlated significantly with
587 the copies of *Variovorax asfA*. This suggests that nematode grazing may significantly affect bacteria-
588 mediated desulfonation of organo-S forms in soil similar to the manner in which they affect organic P
589 transformation in soil. However, the presented data do not allow conclusions on whether the bacterial
590 feeding nematodes selectively feed on desulfonating bacteria. Since *asfA* copies of *Variovorax* were
591 highest in the OF treatment but relative abundance of 16S rRNA copies of *Variovorax* was similar
592 across the treatments, one may speculate that the present nematodes were not selectively feeding on
593 *Variovorax*.

594 In the present study, some of the bacterial genera (based on 16S rRNA sequencing) with
595 significantly higher relative abundance in the OF than in the IF treatment have been reported to be
596 involved in nutrient cycling and/or plant growth promotion. Strains of *Burkholderia* have been reported
597 to be involved in P mineralization (Istina et al. 2015), N cycling and plant growth promotion (Estrada-
598 De Los Santos et al. 2001). Strains of *Allorhizobium*, a member of the rhizobia group (Delgado et al.
599 2007), *Terrimonas* and *Chryseolinea* (Visioli et al. 2018) are involved in N fixation and enhancing
600 nutrient uptake. In addition, strains of *Terrimonas* have been associated with arysulfatase activity
601 (Acosta-Martínez et al. 2014). *Ohtaekwangia* strains have been reported to be involved in plant defense
602 (Visioli et al. 2018) while *Pelomonas* strains have been previously identified as important members of
603 *phoD*-harbouring bacteria (Bi et al. 2019).

604 While the use of molecular tools has tremendously advanced our understanding of the microbial
605 world, the present study also clearly showcases its limitations and the benefit of having cultivation-
606 dependent and microscopy data in order to get a more detailed picture. The present investigation of
607 functional gene abundances showed clear limitations in the ability to universally amplify genes
608 involved in P and S mobilization. However, the described procedure of nematode sequencing has also
609 demonstrated the advancements that can be made using molecular tools alongside analysis via
610 microscopy. In this study, a large number of nematode sequence reads was detected (58 %) with the
611 use of primers designed by Porazinska et al. (2009). This may be attributable to the fact that the

612 nematodes were first extracted and concentrated on membrane filters before nematode DNA extraction.
613 In contrast, Singh et al. (2019) used the same primers but only 8.9 % of the detected reads in their study
614 belonged to nematodes as they used the Baermann funnel and sugar flotation extraction method. The
615 increased abundance of bacterial-feeding nematodes (particularly members of the Rhabditidae family)
616 agrees with the findings of Ferris et al. (2004). These nematode groups are known to increase in
617 abundance in response to the application of organic matter such as manures and crop residues. The
618 feeding type compositions of the nematode faunal assemblage based on both morphological
619 identification (microscopy) and molecular technique (amplicon sequencing) followed similar patterns.
620 This is a significant contribution towards the use of molecular technique in nematode identification as
621 the choice of sample processing techniques seems to play a vital role.

622 Our study reports on the use of animal manure as source of both P and S for grasslands in a
623 relatively short term whereas many previous studies have focused on the use of manure as a source of
624 C, N and/or P (Marschner et al. 2003; Dordas et al. 2008; Fox et al. 2017; Lori et al. 2017; Shi et al.
625 2018; Chen et al. 2018; Martínez-García et al. 2018). It also provided detailed information on the
626 effects of organic versus inorganic fertilizer application on the prokaryotic and eukaryotic microbes as
627 well as nematodes (abundance, community composition, and activity) involved in P and S cycling and
628 grass growth. One limitation of the present study is that repacked soil columns were used. The
629 repacking of the columns is changing some of the soil environmental conditions and the use of columns
630 is affecting the soil temperature when compared to field studies. However, the use of columns enabled
631 the study to take measurements that would have been otherwise very difficult to obtain such as soil
632 solution from different depths.

633 In conclusion, this study has demonstrated that OF application positively impacts most of the
634 investigated microbial, nematode and soil enzymatic parameters even though there was no relative
635 grass growth promotion with the use of the OF. A conceptual model of the OF/IF fertilization has been
636 established (Figure 5) to visualize the outcomes of the fertilizations chosen. Organic fertilizers are
637 applied in most arable systems according to their N content. Therefore, to obtain plant growth
638 promotion with the use of organic fertilizers as a source of P and S, there may be need to apply the
639 organic fertilizer in combination with a readily available source of N like urea when C/N ratios are
640 high. Likewise, the timing of the application needs to be optimized in order to ensure optimal N supply.
641 However, further research is needed on the potential negative effect of nitrate concentrations in soil
642 solution on root mycorrhization and bacterial feeding nematode abundance in order to better manage
643 a potential negative feedback through the addition of N fertilizers to slurry applications. In order to

maximize the benefits of manure-based fertilization, while minimizing any negative impacts e.g. distribution of antibiotic resistance genes, pathogens and heavy metals, a more holistic approach to organic fertilization may be helpful. This could be based in improved animal welfare to lower heavy metal intake and a departure from routine use of antibiotics or could include manure processing such as anaerobic digestion to advance nutrient availability and exclude harmful pathogens.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abubaker J, Risberg K, Pell M (2012) Biogas residues as fertilisers–Effects on wheat growth and soil microbial activities. Appl Energy 99**:** 126-134

Acosta-Martínez V, Cotton J, Gardner T, Moore-Kucera J, Zak J, Wester D, Cox S (2014) Predominant bacterial and fungal assemblages in agricultural soils during a record drought/heat wave and linkages to enzyme activities of biogeochemical cycling. Appl Soil Ecol 84:69–82

Andrássy I (1984) Klasse Nematoda:(Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida). Akademie-Verlag, Berlin.

Apel AK, Sola-Landa A, Rodríguez-García A, MartíN JF (2007) Phosphate control of *phoA*, *phoC* and *phoD* gene expression in *Streptomyces coelicolor* reveals significant differences in binding of PhoP to their promoter regions. Microbiology 153**:** 3527-3537

Bardgett RD, Griffiths BS (1997) Ecology and biology of soil protozoa, nematodes and microarthropods. In Van Elsas J, Trevors J, Wellington EW (Eds) Modern Soil Microbiology. Marcel Dekker, New York, pp 129-164.

Baum C, Makeschin F (2000) Effects of nitrogen and phosphorus fertilization on mycorrhizal formation of two poplar clones (*Populus trichocarpa* and *P. tremula* x *tremuloides*). J Plant Nutr Soil Sci 163**:** 491-497

Bentivenga S, Hetrick B (1992) The effect of prairie management practices on mycorrhizal symbiosis. Mycologia 84: 522-527

Bi QF, Li KJ, Zheng BX, Zheng BX, Liu XP, Li HZ, Jin BJ, Ding K, Yang XR, Lin XY, Zhu YG (2019) Partial replacement of inorganic phosphorus (P) by organic manure reshapes phosphate mobi-lizing bacterial community and promotes P bioavailability in a paddy soil. Sci Total Environ 139477: 10.1016/j.scitotenv.2019.134977

Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Caporaso JG (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6: 90

Bolyen E, Rideout JR, Dillon MR et al. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37: 852-857

Bongers T (1988) De nematoden van Nederland; een identificatietabel voor de in Nederland aangetroffen zoetwater-en bodembewonende nematoden.

Cai F, Pang G, Li R-X, Li R, Gu X-L, Shen Q-R, Chen W (2017) Bioorganic fertilizer maintains a more stable soil microbiome than chemical fertilizer for monocropping. Biol Fertil Soils 53: 861-872

Calderon FJ, Mccarty GW, Van Kessel JAS, Reeves JB (2004) Carbon and nitrogen dynamics during incubation of manured soil. Soil Sci Soc Am J 68**:** 1592-1599

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13: 581- 583

Chardon W, Aalderink G, Van Der Salm C (2007) Phosphorus leaching from cow manure patches on soil columns. J Environ Qual 36**:** 17-22

Chee-Sanford JC, Mackie RI, Koike S, Krapac IG, Lin YF, Yannarell AC, Maxwell S, Aminov RI (2009) Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J Environ Qual 38:1086-1108

Chen Y, Camps-Arbestain M, Shen Q, Singh B, Cayuela ML (2018) The long-term role of organic amendments in building soil nutrient fertility: a meta-analysis and review. Nutr Cycl Agroecosys 111:103-125

Chhabra S, Brazil D, Morrissey J, Burke J, O'Gara F, Dowling DN (2013) Fertilization management affects the alkaline phosphatase bacterial community in barley rhizosphere soil. Biol Fertil Soils 49**:** 31-39

Cleveland CC, Liptzin, D (2007) C: N: P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85**:** 235-252

Cordell D, Drangert JO, White S (2009) The story of phosphorus: global food security and food for thought. Glob Environ Change 19: 292-305

Delgado MJ, Casella S, Bedmar EJ (2007) Denitrification in rhizobia-legume symbiosis. In Bothe H, Ferguson SJ, Newton WE (Eds) Biology of the Nitrogen Cycle. Elsevier, Amsterdam, pp 3-20

Dordas CA, Lithourgidis AS, Matsi T, Barbayiannis N (2008) Application of liquid cattle manure and inorganic fertilizers affect dry matter, nitrogen accumulation, and partitioning in maize. Nutr Cycl Agroecosys 80: 283-296

Elser JJ (2012) Phosphorus: a limiting nutrient for humanity? Curr Opin Biotechnol 23: 833-838

Estrada-De Los Santos P, Bustillos-Cristales R, Caballero-Mellado J (2001) *Burkholderia*, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. Appl Environ Microbiol 67 :2790-2798

Fageria N, Baligar V (2005) Enhancing nitrogen use efficiency in crop plants. Adv Agron 88**:** 97-185

Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biol Cons 61: 1-10

Ferris H, Venette RC, Scow KM (2004) Soil management to enhance bacterivore and fungivore nematode populations and their nitrogen mineralization function. Appl Soil Ecol 24: 19-35

Forge T, Bittman S, Kowalenko C (2005) Responses of grassland soil nematodes and protozoa to multi-year and single-year applications of dairy manure slurry and fertilizer. Soil Biol Biochem 37**:** 1751-1762

Fowler D, Smith R, Muller J, Hayman G, Vincent K (2005) Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. Environ Pollut 137**:** 15-25

Fox A, Ikoyi I, Creamer R, Lanigan G, Schmalenberger A (2017) Microbial community structure and function respond more strongly to temporal progression than to the application of slurry in an Irish grassland. Appl Soil Ecol 120: 97-104

Fox A, Kwapinski W, Griffiths BS, Schmalenberger A (2014) The role of sulfur-and phosphorus-mobilizing bacteria in biochar-induced growth promotion of Lolium perenne. FEMS Microbiol Ecol 90**:** 78-91

Fraser T, Lynch DH, Entz MH, Dunfield KE (2015a) Linking alkaline phosphatase activity with bacterial *phoD* gene abundance in soil from a long-term management trial. Geoderma 257**:** 115-122

Fraser TD, Lynch DH, Bent E, Entz MH, Dunfield KE (2015b) Soil bacterial *phoD* gene abundance and expression in response to applied phosphorus and long-term management. Soil Biol Biochem 88**:** 137-147

Fraser TD, Lynch DH, Gaiero J, Khosla K, Dunfield KE (2017) Quantification of bacterial non-specific acid (*phoC*) and alkaline (*phoD*) phosphatase genes in bulk and rhizosphere soil from organically managed soybean fields. Appl Soil Ecol 111**:** 48-56

Gandhi NU, Chandra SB (2012) A comparative analysis of three classes of bacterial non-specific acid phosphatases and archaeal phosphoesterases: evolutionary perspective. *Acta Inform Med* 20: 167

Gansauge MT, Meyer M (2013) Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA. Nat Protoc 8: 737-748

Gebremikael MT, Steel H, Buchan D, Bert W, De Neve S (2016) Nematodes enhance plant growth and nutrient uptake under C and N-rich conditions. Sci Rep 6**:** 32862

741 Griffiths BS, Wheatley R, Olesen T, Henriksen K, Ekelund F, Rønn R (1998) Dynamics of nematodes and protozoa
742 following the experimental addition of cattle or pig slurry to soil. Soil Biol Biochem 30**:** 1379-1387

743 Harris J, Tyrrel S, Ritz K, Lanigan G, Griffiths B, Brennan F, Bourdin F, Massey P, Moynihan E, Rogers N (2011) Does
744 soil biology hold the key to optimized slurry management? A manifesto for research. Soil Use Manage 27**:** 464-469

745 Hartman WH, Richardson CJ (2013) Differential nutrient limitation of soil microbial biomass and metabolic quotients
746 (qCO2): is there a biological stoichiometry of soil microbes? PloS One 8**:** e57127

747 Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rev
748 Ecol Evol Syst 36**:** 191-218

749 Haynes R, Williams P (1993) Nutrient cycling and soil fertility in the grazed pasture ecosystem. Adv Agron 49: 119-199

750 Hejna M, Gottardo D, Baldi A, Dell'Orto V, Cheli F, Zaninelli M, Rossi L (2018) Review: Nutritional ecology of heavy
751 metals. Animal 12:2156-2170

752 Holden N, Fitzgerald D, Ryan D, Tierney H, Murphy F (2004) Rainfall climate limitation to slurry spreading in Ireland.
753 Agric For Meteorol 122**:** 207-214

754 Ikoyi I, Fowler A, Schmalenberger A (2018) One-time phosphate fertilizer application to grassland columns modifies the
755 soil microbiota and limits its role in ecosystem services. Sci Total Environ 630**:** 849-858

756 Ikoyi I, Fowler A, Storey S, Doyle E, Schmalenberger A (2019) Sulfate fertilization supports growth of ryegrass in soil
757 columns but changes microbial community structures and reduces abundances of nematodes and arbuscular
758 mycorrhiza. Sci Total Environ 135315: 10.1016/j.scitotenv.2019.135315

759 Ingham E, Trofymow J, Ames R, Hunt H, Morley C, Moore J, Coleman D (1986) Trophic interactions and nitrogen cycling
760 in a semi-arid grassland soil. II. System responses to removal of different groups of soil microbes or fauna. J Appl
761 Ecol 23**:** 615-630

762 Ingham RE, Trofymow J, Ingham ER, Coleman DC (1985) Interactions of bacteria, fungi, and their nematode grazers:
763 effects on nutrient cycling and plant growth. Ecol Monogr 55**:** 119-140

764 Irshad U, Villenave C, Brauman A, Plassard C (2011) Grazing by nematodes on rhizosphere bacteria enhances nitrate and
765 phosphorus availability to *Pinus pinaster* seedlings. Soil Biol Biochem 43**:** 2121-2126

766 Istina IN, Widiastuti H, Joy B, Antralina M (2015) Phosphate solubilizing microbe from Saprists peat soil and their potency
767 to enhance oil palm growth and P uptake. Proc Food Sci 3: 426-435

768 Jairajpuri MS, Ahmad W (1992) Dorylaimida: free-living, predaceous and plant-parasitic nematodes. Oxford and IBH
769 Publishing Co., Delhi.

770 Jiang Y, Liu M, Zhang J, Chen Y, Chen X, Chen L, Li H, Zhang XX, Sun B (2017) Nematode grazing promotes bacterial
771 community dynamics in soil at the aggregate level. ISME J 11: 2705-2717

772 Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on
773 fast Fourier transform. Nucl Acids Res 30: 3059-3066

774 Kertesz MA, Mirleau P (2004) The role of soil microbes in plant sulphur nutrition. J Exp Bot 55: 1939-1945

775 Kircher M, Sawyer S, Meyer M (2012) Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina
776 platform. Nucl Acids Res 40: e3

777 Lidbury IDEA, Murphy ARJ, Scanlan DJ, Bending GD, Jones AME, Moore JD, Goodall A, Hammond JP, Wellington
778 EMH (2016) Comparative genomic, proteomic and exoproteomic analyses of three *Pseudomonas* strains reveals
779 novel insights into the phosphorus scavenging capabilities of soil bacteria. Environ Microbiol 18: 3535-3549

780 Lori M, Symnaczik S, Mäder P, De Deyn G, Gattinger A (2017) Organic farming enhances soil microbial abundance and
781 activity—A meta-analysis and meta-regression. PloS One 12**:** e0180442

782 Lozupone CA, Hamady M, Kelley ST, Knight R (2007) Quantitative and qualitative beta diversity measures lead to
783 different insights into factors that structure microbial communities. Appl Environ Microbiol 73: 1576-1585

784 Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ
785 Microbiol 71: 8228- 8235

786 Marschner P, Kandeler E, Marschner B (2003) Structure and function of the soil microbial community in a long-term
787 fertilizer experiment. Soil Biol Biochem 35: 453-461

788 Martínez-García LB, Korthals G, Brussaard L, Jørgensen HB, De Deyn GB (2018) Organic management and cover crop
789 species steer soil microbial community structure and functionality along with soil organic matter properties. Agric
790 Ecosyst Environ 263**:** 7-17

791 Matsi T, Lithourgidis AS, Gagianas AA (2003) Effects of injected liquid cattle manure on growth and yield of winter wheat
792 and soil characteristics. Agron J 95**:** 592-596

793 McCormack S (2002) Analysis of agricultural materials: methods used at Johnstown Castle Research Centre. Teagasc,
794 Johnstown Castle Environment Research Centre, Wexford, Ireland.

795 McGonigle T, Miller M, Evans D, Fairchild G, Swan J (1990) A new method which gives an objective measure of
796 colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol 115: 495-501

797 Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal
798 Chim Acta 27**:** 31-36

799 Myrold D (1999) Transformation of nitrogen. In: Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (Eds) Principles and
800 applications of soil microbiology, Pearson Prentice Hall Upper Saddle River, NJ, 550

801 Neher DA (1999) Nematode communities in organically and conventionally managed agricultural soils. J Nematol 31**:** 142-
802 154.

803 Neufeld KR, Grayston SJ, Bittman S, Krzic M, Hunt DE, Smukler SM (2017) Long-term alternative dairy manure
804 management approaches enhance microbial biomass and activity in perennial forage grass. Biol Fertil Soils 53: 613-
805 626

806 Noll M, Wellinger M (2008) Changes of the soil ecosystem along a receding glacier: testing the correlation between
807 environmental factors and bacterial community structure. Soil Biol Biochem 40**:** 2611-2619

808 O'Mara F (2008) Country pasture/forage resource profile/Ireland. FAO of the UN, Rome.

809 Okada H, Oba H (2008). Comparison of nematode community similarities assessed by polymerase chain reaction-
810 denaturing gradient gel electrophoresis (DGGE) and by morphological identification. Nematology 10 689-700

811 Pascual J, Garcia C, Hernandez T, Moreno J, Ros M (2000) Soil microbial activity as a biomarker of degradation and
812 remediation processes. Soil Biol Biochem 32: 1877-1883

813 Peech M, English L (1944) Rapid microchemical soil tests. Soil Sci 57: 167-196

814 Porazinska DL, Giblin-Davis RM, Faller L, Farmerie W, Kanzaki N, Morris K, Powers TO, Tucker AE, Sung W, Thomas
815 WK (2009) Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity.
816 Mol Ecol Resour 9: 1439-1450

817 Price MN, Dehal PS, Arkin AP (2010) FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS
818 One 5: e9490

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41: D590-D596

Ragot SA, Kertesz MA, Bünemann EK (2015) Diversity of the *phoD* alkaline phosphatase gene in soil. Appl Environ Microbiol 81**:** 7281-7289

Sakurai M, Wasaki J, Tomizawa Y, Shinano T, Osaki M (2008) Analysis of bacterial communities on alkaline phosphatase genes in soil supplied with organic matter. Soil Sci Plant Nutr 54: 62-71

Schmalenberger A, Noll M (2014) Bacterial communities in grassland turfs respond to sulphonate addition while fungal communities remain largely unchanged. Eur J Soil Biol 61**:** 12-19

Schmalenberger A, Hodge S, Bryant A, Hawkesford MJ, Singh BK, Kertesz MA (2008) The role of *Variovorax* and other *Comamonadaceae* in sulfur transformations by microbial wheat rhizosphere communities exposed to different sulfur fertilization regimes. Environ Microbiol 10**:** 1486-1500

Shi Y, Ziadi N, Hamel C, Bittman S, Hunt D, Lalande R, Shang J (2018) Soil microbial biomass, activity, and community composition as affected by dairy manure slurry applications in grassland production. Appl Soil Ecol 125**:** 97-107

Siddiqi MR (1986) Tylenchida: parasites of plants and insects. Commonwealth Agricultural Bureaux

Sieh D, Watanabe M, Devers EA, Brueckner F, Hoefgen R, Krajinski F (2013) The arbuscular mycorrhizal symbiosis influences sulfur starvation responses of *Medicago truncatula*. New Phytol 197**:** 606-616

Singh D, Slik JF, Jeon YS, Tomlinson KW, Yang X, Wang J, Kerfahi D, Porazinska DL, Adams JM (2019) Tropical forest conversion to rubber plantation affects soil micro-& mesofaunal community & diversity. Sci Rep 9**:** 5893

Smith SE, Smith FA (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. Mycologia 104**:** 1-13

Steiner C, Teixeira WG, Lehmann J, Nehls T, De Macêdo JLV, Blum WE, Zech W (2007) Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant Soil 291**:** 275-290

Tabatabai M, Bremner J (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1**:** 301-307

Tabatabai M, Bremner J (1970a) Arylsulfatase activity of soils. Soil Sci Soc Am J 34**:** 225-229

Tabatabai M, Bremner J (1970b) Factors affecting soil arylsulfatase activity. Soil Sci Soc Am J 34**:** 427-429

Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M (2014) Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using Next-Generation Sequencing. PLOS ONE 9:e105592

Taupe N (2016) Thermal treatment technologies for low moisture and dehydrated manure feedstock. Thesis (PhD), University of Limerick, URI: http://hdl.handle.net/10344/7113

Vance CP (2001) Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiol 127**:** 390-397

Visioli G, Sanangelantoni AM, Vamerali T, Dal Cortivo C, Blandino M (2018) 16S rDNA profiling to reveal the influence of seed-applied biostimulants on the rhizosphere of young maize plants. Molecules 23: 1-18

Wei X, Hu Y, Razavi BS, Zhou J, Shen J, Nannipieri P, Wu J, Ge T (2019) Rare taxa of alkaline phosphomonoesterase-harboring microorganisms mediate soil phosphorus mineralization. Soil Biol Biochem 131:62-70

Whitehead A, Hemming J (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annal Appl Biol 55**:** 25-38

858 Williams PH, Haynes RJ (1993) Forms of sulphur in sheep excreta and their fate after application on to pasture soil. J Sci
859 Food Agric 62: 323-329

860 Xue C, Penton CR, Zhu C, Chen H, Duan Y, Peng C, Guo S, Ling N, Shen Q (2018) Alterations in soil fungal community
861 composition and network assemblage structure by different long-term fertilization regimes are correlated to the soil
862 ionome. Biol Fertil Soils 54:95

863 Zhang Y, Shen H, He X, Thomas BW, Lupwayi NZ, Hao X, Thomas MC, Shi X (2017) Fertilization shapes bacterial
864 community structure by alteration of soil pH. Front Microbiol 8: 1325

865 Zhao J, Wang F, Li J, Zou B, Wang X, Li Z, Fu S (2014) Effects of experimental nitrogen and/or phosphorus additions on
866 soil nematode communities in a secondary tropical forest. Soil Biol Biochem 75**:** 1-10

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868 **Table 1**

869 Mass balance: mean total grass shoot dry weight (g per column; n=6) ± standard error and mean grass
870 nutrient content (mg per column). Different letters in the same row indicate significant difference
871 (P<0.05).

	Urea only Control (UC)	Inorganic Fertilizer (IF)	Organic Fertilizer (OF)
Shoot dry weight	$23.1^{b} \pm 0.66$	$26.3^{b} \pm 1.72$	$11.8^{c} \pm 0.64$
Calcium	134.0^{b}	169.5^{a}	81.4^{c}
Magnesium	56.7^{b}	75.9^{a}	30.3^{c}
Sulphur	26.9^{b}	94.4^{a}	38.5^{b}
Nitrogen	174.6^{b}	261.2^{a}	111.5^{c}
Phosphorus	41.9^{b}	53.6^{a}	38.9^{b}
Potassium	443.7^{b}	750.5^{a}	284.2^{c}
Boron	0.19^{a}	0.23^{a}	0.08^{b}
Copper	0.12^{a}	0.14^{a}	0.04^{b}
Molybdenum	0.10^{a}	0.06^{b}	0.05^{b}
Iron	2.25^{a}	1.51^{a}	1.62^{a}
Zinc	0.28^{b}	0.35^{a}	0.18^{c}
Manganese	0.79^{ab}	0.52^{b}	1.14^{a}

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Table 2

MPN values of P and S mobilizing bacteria (MM2Phy, MM2PAA, MM2TS), total heterotrophic bacteria (R2A), CFU values of tri-calcium phosphate solubilizing (TCP) bacteria, total nematode abundance (TNEM) and abundance of bacterial-feeding (BF) and fungal-feeding (FF) nematodes, arbuscular (AC) and hyphal colonization (HC) rates and vesicles (VC) of ryegrass roots, alkaline (ALP) and acid phosphomonoesterase (ACP) activities, arylsulfatase activity (ASULF) as well as bacterial *phoD*, *phoC* and *asfA* gene copies number per gram of soil determined via qPCR. Different letters in the same row indicate significant differences ($P < 0.05$); ± = standard error; n=6.

	Urea only Control (UC)	Inorganic Fertilizer (IF)	Organic Fertilizer (OF)
MM2Phy	$3.5 \times 10^{7a} \pm 1.0 \times 10^{7}$	$2.3 \times 10^{7a} \pm 6.4 \times 10^{6}$	$6.0 \times 10^{7a} \pm 3.5 \times 10^{7}$
MM2PAA	$2.4 \times 10^{7ab} \pm 6.4 \times 10^{6}$	$1.0 \times 10^{7b} \pm 1.8 \times 10^{6}$	$2.9 \times 10^{7a} \pm 8.9 \times 10^{6}$
MM2TS	$2.3 \times 10^{7b} \pm 2.3 \times 10^{6}$	$1.3 \times 10^{7b} \pm 4.0 \times 10^{6}$	$5.4 \times 10^{7a} \pm 2.3 \times 10^{7}$
TCP	$1.0 \times 10^{6a} \pm 1.2 \times 10^{5}$	$7.0 \times 10^{5a} \pm 6.8 \times 10^{4}$	$8.8 \times 10^{6a} \pm 1.1 \times 10^{5}$
R2A	$1.3 \times 10^{8b} \pm 2.6 \times 10^{7}$	$1.2 \times 10^{8b} \pm 3.1 \times 10^{7}$	$6.2 \times 10^{8a} \pm 3.5 \times 10^{8}$
TNEM (No./100g soil)	$340^{b} \pm 43$	$276^{b} \pm 57$	$482^{a} \pm 67$
BF (No./100g soil)	$167^{b} \pm 12$	$82^{c} \pm 33$	$279^{a} \pm 48$
FF (no./100g soil)	$19^{a} \pm 5$	$21^{a} \pm 5$	$32^{a} \pm 9$
AC (%)	$79.7^{a} \pm 9.6$	$52.5^{b} \pm 7.5$	$86.7^{a} \pm 5.9$
VC (%)	$23.6^{a} \pm 9.3$	$8.6^{b} \pm 2.5$	$30.3^{ab} \pm 5.0$

HC (%)	$84.7^{a} \pm 7.6$	$54.7^{b} \pm 6.9$	$90.0^{a} \pm 5.7$
ALP(µgPNP/g soil/hr)	$450^{b} \pm 58$	$502^{ab} \pm 87$	$642^{a} \pm 147$
ACP(µgPNP/g soil/hr)	$627^{b} \pm 61$	$731^{a} \pm 33$	$688^{ab} \pm 36$
ASULF (µgPNP/g soil/hr)	$384^{b} \pm 47$	$399^{b} \pm 57$	$525^{a} \pm 83$
Morgan's P (mg/L)	$2.47^{b} \pm 0.15$	$2.57^{b} \pm 0.17$	$4.06^{a} \pm 1.08$
Soil pH	7.40^{c}	7.83^{b}	8.39^{a}
phoD	$3.29 \times 10^{5a} \pm 4.0 \times 10^{4}$	$3.28 \times 10^{5a} \pm 3.3 \times 10^{4}$	$3.33 \times 10^{5a} \pm 4.9 \times 10^{4}$
PhoC	$2.66 \times 10^{5a} \pm 5.2 \times 10^{4}$	$2.69 \times 10^{5a} \pm 3.3 \times 10^{4}$	$2.98 \times 10^{5a} \pm 4.1 \times 10^{4}$
Variovorax asfA	$1.6 \times 10^{5b} \pm 2.2 \times 10^{4}$	$1.9 \times 10^{5b} \pm 3.4 \times 10^{4}$	$4.8 \times 10^{5a} \pm 7.3 \times 10^{4}$
Burkholderia asfA	$1.8 \times 10^{6a} \pm 2.7 \times 10^{5}$	$2.0 \times 10^{6a} \pm 1.9 \times 10^{5}$	$1.2 \times 10^{6a} \pm 2.7 \times 10^{5}$
Polaromonas asfA	$1.2 \times 10^{5a} \pm 2.7 \times 10^{4}$	$1.8 \times 10^{5a} \pm 4.7 \times 10^{4}$	$2.1 \times 10^{5a} \pm 4.6 \times 10^{4}$

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Figure captions:

1. Mean nutrient concentrations in soil columns at 0-20 cm: a) phosphate b) sulfate and c) nitrate in soil solution. UC = urea only control, IF = inorganic fertilizer, OF = organic fertilizer, error bars = standard error of means, n = 6.

2. CCA biplots of PCR-DGGE fingerprint showing the effects of treatments on a) bacterial 16S rRNA gene b) bacterial *phoD* gene c) fungal ITS d) AM fungal 18S rRNA and e) nematode 18S rRNA gene with bacterial-feeding nematodes abundance (BF), fungal-feeding (FF) nematode abundance, *Variovorax asfA* gene abundance (Var), Morgan's P (AvP), ALP, arylsulfatase (ASULF), ACP, total nematode abundance (TNEM), total grass shoot dry weight (TDWT), pH, shoot N, P and S contents, abundance of cultivable calcium-phosphate solubilizing bacteria (TCP), phosphonoacetic acid utilizing (PAA), phytate-utilizing (PHY), total heterotrophic (R2A) bacteria, and percentage of root colonized by mycorrhizal arbuscules, vesicles and hyphae (AC, VC, HC) defined as environmental factors. Black circles = urea-only control (UC), red squares = inorganic fertilizer (IF), and green triangles = organic fertilizer (OF). Arrows for each variable tested denote significant correlation ($p \leq 0.05$, permutation test) of environmental factors with shift of the community composition.

3. a) Non-metric multidimensional scaling (NMDS) biplot of the bacterial community sequences based on 16S rRNA genes; circles = urea-only control (control), squares = inorganic fertilizer (inorganic), triangles = organic fertilizer (organic); n=6. b) Bar plot of the mean percentage relative abundances of the dominant significantly different genera based on the 16S rRNA gene sequence analyses. Red bars = inorganic fertilizer (IF) and green bars = organic fertilizer (OF); error bars = standard error of the means

4. Non-metric multidimensional scaling (NMDS) biplot of the nematode community sequences based on 18S rRNA genes; circles = urea only control (control), squares = inorganic fertilizer (inorganic), triangles = organic fertilizer (organic); n=6.

5. Conceptual model of Phosphorus (P) and Sulfur (S) cycling in the soil columns with organic (O, dark brown) and inorganic (I, light blue) fertilizer and its effect on the soil biota; upwards arrows indicate an increase, while downwards arrows indicate a decrease; horizontal arrows indicate a change or shift.

926 Figure 1

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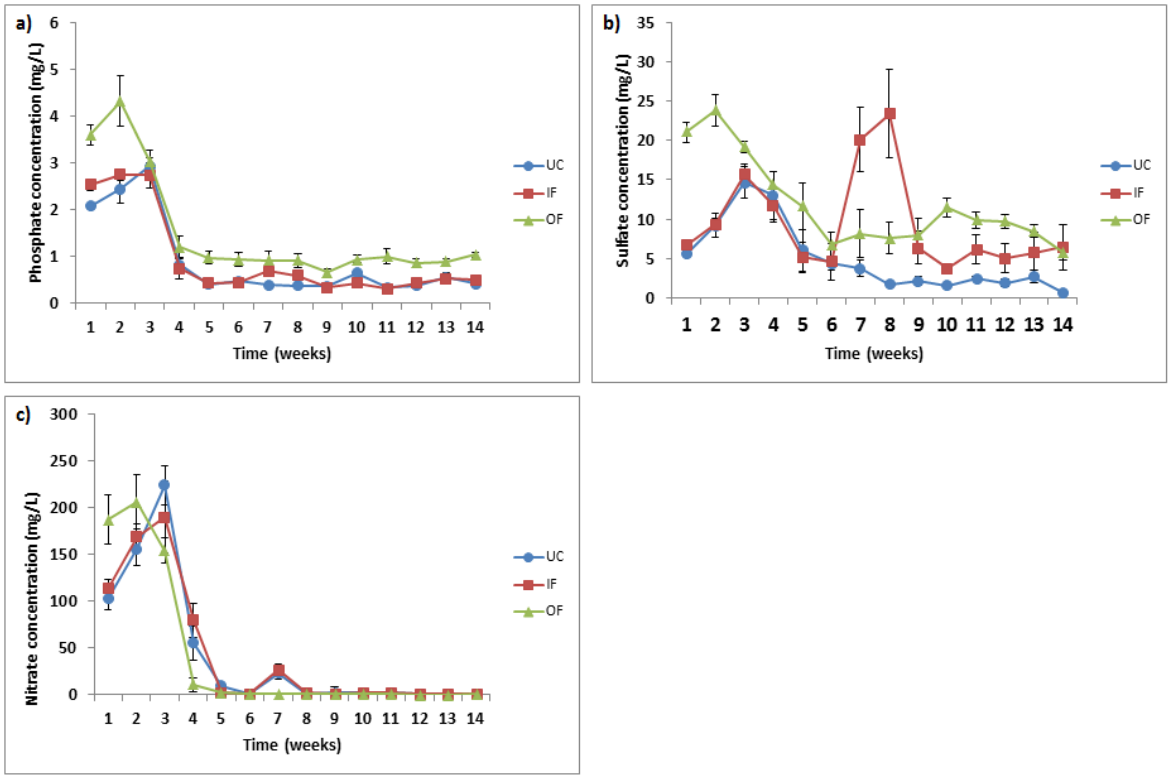

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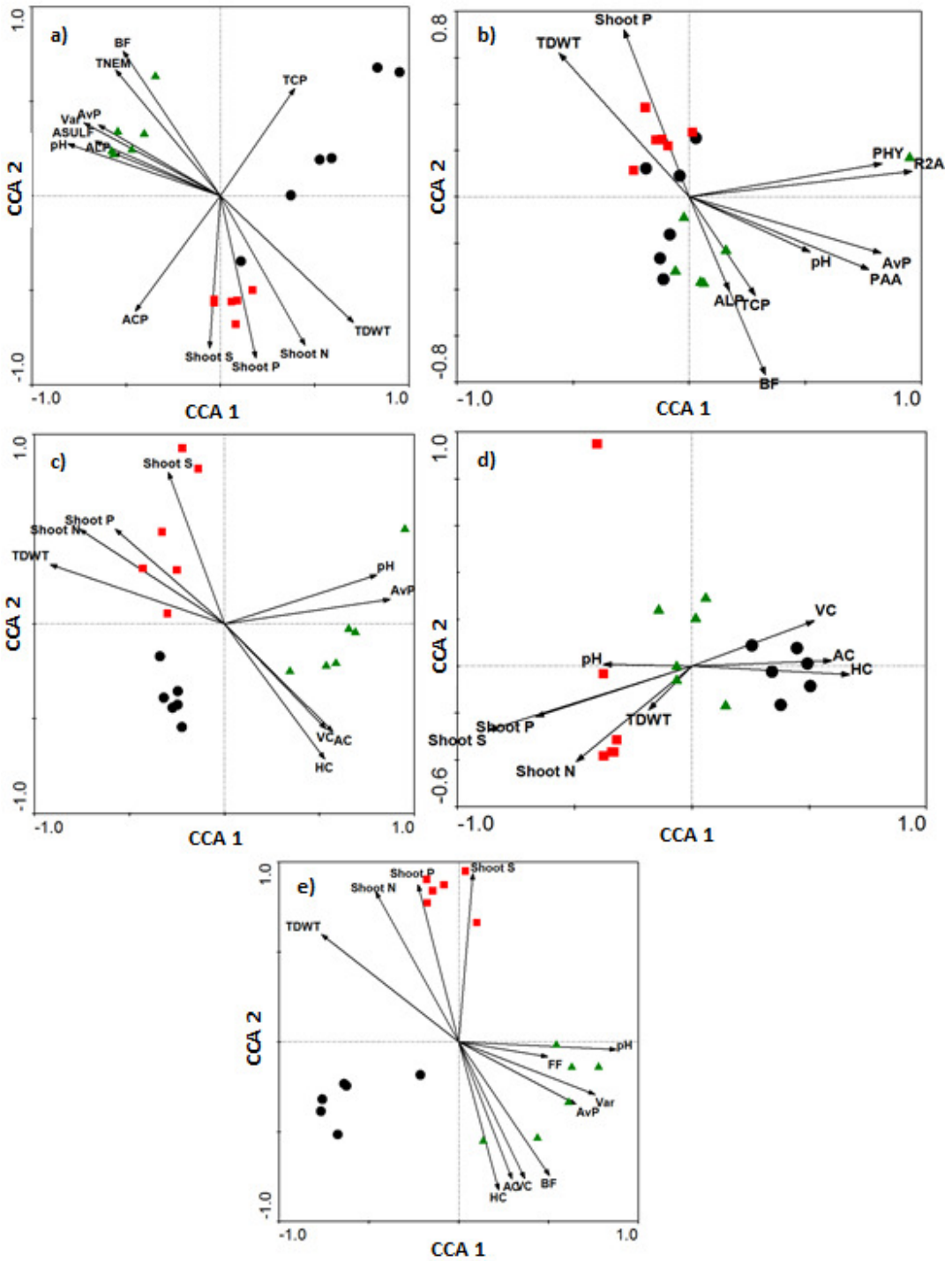
936 Figure 2



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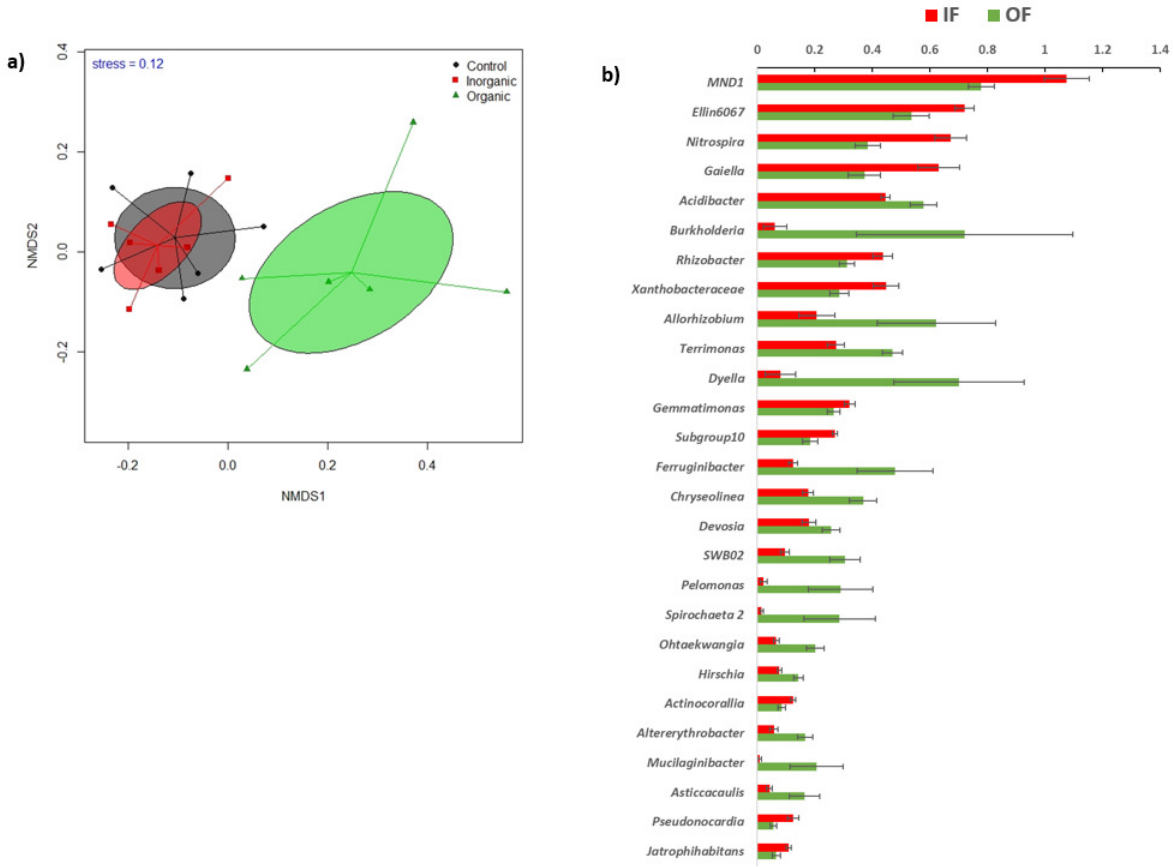
942 Figure 3



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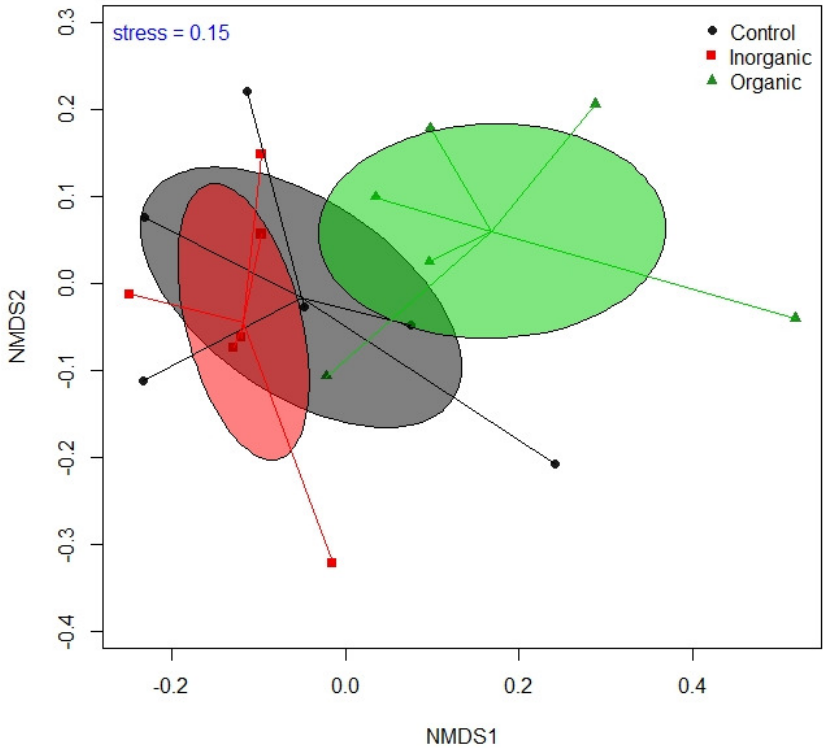
947 Figure 4



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949 Figure 5

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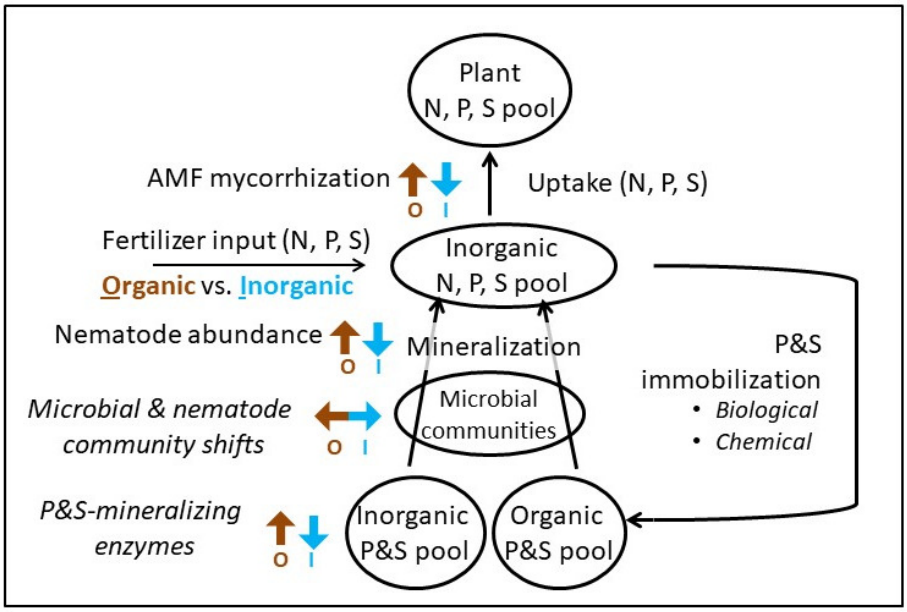

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