CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Manipulation of sward diversity is a more effective management strategy than addition of microbial inoculants in intensively managed grassland
Authors	Ikoyi, Israel;Duff, Aoife M.;Finn, John A.;Wall, David P.;Kostic, Tanja;Lucic-Mercy, Eva;Sessitsch, Angela;Brennan, Fiona
Publication date	2024-07-24
Original Citation	Ikoyi, I., Duff, A. M., Finn, J. A., Wall, D. P., Kostic, T., Lucic-Mercy, E., Sessitsch, A. and Brennan, F. (2024) 'Manipulation of sward diversity is a more effective management strategy than addition of microbial inoculants in intensively managed grassland', Plant and Soil, 509(1), pp. 139-156. https://doi.org/10.1007/s11104-024-06849-7
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1007/s11104-024-06849-7
Rights	© 2024, the Authors. Open Access. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/ - https://creativecommons.org/licenses/by/4.0/
Download date	2026-11-05 23:59:26
Item downloaded from	https://hdl.handle.net/10468/16180



University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Plant Soil
https://doi.org/10.1007/s11104-024-06849-7

RESEARCH ARTICLE

Manipulation of sward diversity is a more effective management strategy than addition of microbial inoculants in intensively managed grassland

Israel Ikoyi · Aoife M. Duff · John A. Finn · David P. Wall · Tanja Kostic · Eva Lucic-Mercy · Angela Sessitsch · Fiona Brennan

Received: 8 April 2024 / Accepted: 9 July 2024
© The Author(s) 2024

Abstract

Background and aims Diversifying grassland sward composition and application of microbial inoculants are potential alternative routes to facilitate enhanced nutrient acquisition by plants, but their relative effects have been rarely tested in grasslands. In a two-year field experiment, we investigated the impact of inoculants and sward types on forage yield, nutrient uptake, and the soil microbiome.

Methods We implemented a fully factorial experiment with inoculants (eight levels; arbuscular mycorrhizal fungi (AMF), *Paraburkholderia phytofirmans* PsJN and *Bacillus* sp. P5 (P5), applied singly and in combination), and sward types (three levels: grass-only, grass and legume, mixture of grass, legume, and herb) as the two factors.

Results In both years, there was a very strong effect of sward type, with the grass + legume and grass + legume + herb swards having much higher yields and nutrient uptake (N, P, K, S, Ca, Mg, Zn, Cu) than grass-only. Generally, there was no effect of the inoculants on yield and nutrient uptake, with limited exceptions involving AMF + P5. The best-performing microbial inoculant increased forage yield by 597 kg/ha/year, while switching from grass-only to one of the other sward types increased forage yield by 3932 kg/ha/year (grass + legume) and 4693 kg/ha/year (grass + legume + herb). The inoculants persisted in plots for > 1 year after application. Inoculants and sward type significantly affected the overall prokaryotic and fungal community structures.

Conclusion Overall, under controlled field plot conditions, including legumes and herbs in a grass sward proved to be a far better farm-scale management strategy for increasing grassland forage yield and nutrient uptake than the application of microbial inoculants.

Responsible Editor: Matthias Volk.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-024-06849-7.

I. Ikoyi · A. M. Duff · J. A. Finn · D. P. Wall · F. Brennan
Environment, Soils and Land Use Department, Teagasc, Johnstown Castle, Wexford, Ireland

I. Ikoyi (✉)
School of Biological, Earth and Environmental Sciences, University College Cork, Distillery Fields, North Mall, Cork, Ireland
e-mail: israelikoyi@ucc.ie

I. Ikoyi
Environmental Research Institute, University College Cork, Cork, Ireland

T. Kostic · A. Sessitsch
Bioresources Unit, AIT Austrian Institute of Technology GmbH, Tulln, Austria

E. Lucic-Mercy
Inoq GmbH, Schnega, Germany

Published online: 24 July 2024

Keywords Microbial biofertilizer · Mycorrhiza · Forage swards · *Bacillus* sp. P5 · *Paraburkholderia phytofirmans* PsJN · Grassland sustainability

Introduction

Agricultural production relies heavily on the use of chemical inputs such as fertilizers and pesticides, that can cause negative environmental impacts (poor water quality, increased greenhouse gases, biodiversity loss, lower C sequestration potential). This in turn affects soil health and ultimately the sustainability of the system (Tilman et al. 2002). The delivery of ecosystem services such as clean water, maintenance of soil health for productivity, and the support for biodiversity are the main drivers of sustainability initiatives (Teague and Kreuter 2020).

Plants live in close association and interact with both rhizosphere and endophytic microorganisms. These microorganisms, mainly bacteria and fungi, can be used as inoculants positively affecting the performance and growth of plants, particularly under stress conditions (Benmrid et al. 2023; Cornell et al. 2021). Microbial inoculants that can increase nutrient uptake in plants (biofertilizers) are proposed as a sustainable alternative to chemical fertilizers by utilising the beneficial interactions between plants and specific microorganisms. These bioinoculants mainly include nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and mycorrhizal fungi (Esmaeel et al. 2018; Glick 2004; Marschner and Dell 1994). Benefits of microbial inoculants can include increased nutrient availability, improved soil structure, reduced dependence on synthetic chemical fertilizers, and minimized environmental impact (Schütz et al. 2018). Seed inoculation with beneficial microorganisms has been reported as a promising tool for improving plant growth and development in cereals (Divan Baldani et al. 2000; Galindo et al. 2021; Mitter et al. 2017; Naveed et al. 2014, 2015), for tomatoes (Gravel et al. 2007; Issa et al. 2018; Pillay and Nowak 1997), and other crops (Omirou et al. 2016; Vessey and Buss 2002; Zhang et al. 2019).

Several studies have shown that arbuscular mycorrhizal fungi (AMF) can enhance the absorption of plant nutrients (especially phosphorus), increase drought tolerance, and confer resistance to disease in plants (Augé et al. 2001; Lutz et al. 2023; Newsham et al. 1995; Shi et al. 2023; Walder and van der Heijden 2015). In addition, several strains belonging to the genera *Bacillus* and *Paraburkholderia* (mainly strain PsJN) are well recognized as plant growth promoting bacteria (Bashan and Holguin 1998; Rahman et al. 2018; Sheibani-Tezerji et al. 2015).

There are many proposed bioinoculants, but their use needs to be underpinned by supporting scientific evidence of their effectiveness as biofertilizers. The perennial nature of grasslands with low disturbance and typically high soil C content (which often supports a high diversity of well adapted microbial communities) may make establishment of bioinoculants in grassland more challenging than in other crops. Surprisingly little evidence is available for the effectiveness of the many bioinoculant products that are available for use in grassland systems. Even where there are examples of effective bioinoculant use in cereals, trees, tomatoes, etc., (Li et al. 2022) the realism and farm-scale relevance of the conditions in many experiments can be questioned: often sterilised soil is used; studies are typically limited to pot experiments; experiments are of short duration; conducted in controlled environments (e.g. chambers or glasshouses) that do not necessarily reflect outdoor growing conditions for farmed crops (Bauer et al. 2012; Berruti et al. 2016; Kim et al. 2012; Lekberg and Helgason 2018; Pánková et al. 2018; Wang et al. 2015; Yu et al. 2022). Questions remain regarding the capacity of microbial inoculants to compete for niche space with the locally adapted microbial community and the impacts, if any, that they have on the native soil microbial community (Cornell et al. 2021; O'Callaghan et al. 2022; Trabelsi and Mhamdi 2013).

In intensively managed temperate grassland systems, perennial ryegrass (*Lolium perenne*) is the commonly sown grass species because of its high quantity and quality of forage (Frame and Laidlaw 2011). However, perennial ryegrass systems are heavily dependent on high inputs of inorganic nitrogen (N) fertilizer. This system has become increasingly unsustainable due to multiple reasons that include widespread policy aims and drivers for lower N fertiliser use, increased fertilizer costs, negative environmental impacts, and concerns regarding impacts on soil biodiversity (Bell et al. 2015; Canfield et al. 2010; Reheul et al. 2017). As an alternative, the use of grass/clover and multispecies forage swards have been shown to support high forage yields at similar or even reduced inorganic N input rate (Grange et al. 2021; Moloney et al. 2020), delivering multiple

benefits including enhanced environmental outcomes such as reduced nitrous oxide emissions intensity (Cummins et al. 2021), weed suppression (Connolly et al. 2018; Shnel et al. 2021), drought resilience (Grange et al. 2021) and enhanced biological soil quality (Ikoyi et al. 2023).

As the cost of chemical fertilizers continue to rise and policies related to the reduction in use of chemical fertilizers are enacted, there is a need for practical solutions that can be implemented at farm scale in intensively managed grassland systems. Testing the combination of microbial inoculants with different levels of grassland sward diversity under field conditions is highly novel. Understanding the relative benefits of bioinoculants and sward diversity both separately and in combination is necessary to underpin advice for farm-scale decision-making.

In this study, we implemented a fully factorial experiment in a two-year field experiment with the two factors 'bioinoculants' (singly and in combination) and 'sward diversity', to address the following research questions: 1) What is the effect of bioinoculants, sward diversity and their interaction on yield and nutrient uptake? 2) Do the bioinoculants persist in soils following application? 3) What is the effect of sward type and bioinoculants on the wider soil microbial community diversity and structure?

Materials and methods

Site description, treatments, field establishment and field sampling

A field trial was established in 2020 on a permanent grassland site in the southeast of Ireland located at the Teagasc, Johnstown Castle Research Station (52°17'N; 6°30'W) beef farm. The soil type at this site was a Typic Cambisol with a sandy loam texture and was managed under natural rainfall conditions and according to normal grassland agronomic practice in Ireland. The site was selected because of existing low P levels (Index 1–2, Morgan's P). Prior to the establishment of the experiment the site was sprayed with glyphosate prior to being deep ploughed and power harrowed. The plots were marked out in a randomised complete block design with six replications and a plot size 5 m x 2 m. The seed for each grassland sward type (grass only, grass + legume and grass + legume + herb) were sown by hand on 17th September 2020. After sowing, the area was tine-harrowed to get good soil seed contact for germination, and then rolled to compact the surface and cover the seed.

The eight microbial biofertilizer treatments included a control, AMF and two bacterial strains (*Paraburkholderia phytofirmans* PsJN and *Bacillus* sp. P5) applied either singly or in tandem (Table 1) under three grass sward vegetation types based on the following seed weight proportions: grass-only (100% *Lolium perenne*), grass + legume (70% *Lolium perenne*, 30% *Trifolium repens*) and grass + legume + herb (30% *Lolium perenne*, 25% *Phleum pratense*, 20% *Trifolium repens*, 10% *Trifolium pratense*, 7% *Cichorium intybus*, *and* 8% *Plantago lanceolata*). These microbial strains were selected for their proposed ability to promote plant growth. The plots received 100 kg ha^{-1} yr^{-1} of N in the form of protected urea, split evenly into five applications. Approximately four to six weeks after each fertilizer application, grass was cut to 5 cm, yield was recorded, and all grass removed from the plot. Soil

Table 1 The inoculant treatments applied to each of the three vegetation types

Treatment type	Microbial treatment
1. Control	None
2. AMF	Arbuscular Mycorrhizal Fungi
3. PsJN	*Paraburkholderia phytofirmans* PsJN
4. P5	*Bacillus* sp. P5
5. AMF + PsJN	Arbuscular Mycorrhizal Fungi, *Paraburkholderia phytofirmans* PsJN
6. AMF + P5	Arbuscular Mycorrhizal Fungi, *Bacillus* sp. P5
7. PsJN + P5	*Paraburkholderia phytofirmans* PsJN, *Bacillus* sp. P5
8. AMF + PsJN + P5	Arbuscular Mycorrhizal Fungi, *Paraburkholderia phytofirmans* PsJN, *Bacillus* sp. P5

AMF Arbuscular Mycorrhizal Fungi (mix of *Rhizophagus Irregularis* QS81, *Funneliformis Mosseae* D54 and *Funneliformis caledonium* Ba1)

samples were collected after each harvest as a composite sample (10–15 soil cores) being taken at 0 to 10 cm soil depth along a W pattern across each plot. The cores were placed in sterile plastic bags and homogenised before taking a subsample for molecular analysis, which was immediately flash frozen using liquid nitrogen and stored at -80 °C until further analysis.

Seed preparation

The AMF inoculum was a commercial product (Advantage grade I; 78.5 Mio propagules/kg; INOQ GmbH): Bacterial strains of *P. phytofirmans* PsJN and *Bacillus* sp. P5 were provided by AIT Austrian Institute of Technology and a commercial company, respectively. Cultures of the bacterial strains (*P. phytofirmans* PsJN and *Bacillus* sp. P5) were inoculated in Luria-Bertani (LB) broth and grown aerobically at 28 and 30°C, respectively for 24 h (P5) and 48 h (PsJN). The grown cultures were centrifuged for 20 min at 3000 × g to concentrate the cells. The concentrated cells were re-suspended in sterile phosphate buffered saline (PBS), adjusted to a concentration of 1×10^9 CFU/ml by measuring the absorbance of the sample at 600 nm, and calculating CFU per ml using a standard curve. Bacterial inoculants were added to the weighed seeds for each individual plot and shaken in the New Brunswick Innova 44R incubator (Fisher Scientific, Ireland) for 1 h at 135 RPM at 25°C. Control seeds were soaked in sterile PBS. Excess liquid was removed from the seeds using a sterile syringe and needle. Within two hours of inoculation, the inoculated seeds were taken to the field for sowing on the same day. A subsample of the soaked seeds was taken for DNA extraction and qPCR analysis for the detection of the bacterial strains (see Sect. 2.3 for details on molecular analyses). For the AMF treatments, for each plot, 1 g of AMF was mixed with 100 g of sand and stored in a separate cup to the seed mixture. On the field, the seeds for each plot were mixed with 2 kg of sand (also mixed with the cup containing AMF for AMF treatments) and spread evenly on the plot by hand.

Forage analyses

A composite sample of fresh weight aboveground biomass from each harvest and plot was weighed and dried at 70 °C until it reached a constant weight. Once dried, dry weight biomass was recorded and used to calculate dry matter yield. The dried samples were ground, sieved to 2 mm size and used for nutrient analysis. Total crop P, K, S, Mg, Cu and Ca were analysed using an Agilent 5100 ICP-OES after microwave-assisted acid digestion of the sieved samples (USEPA, 1996). Total N was determined using a combustion analyser (LECO TruSpec CN analyser). Nutrient uptake was calculated as the product of nutrient concentration in yield (%) and total yield.

Molecular analyses

To confirm the inoculation of the treated seeds, DNA was extracted from 0.25 g of inoculated seeds using DNeasy PowerSoil Pro kit (Qiagen, Ireland) as per the manufacturer's instructions. Prior to extraction, the seed samples were blended using a D1000 handheld homogeniser (Sigma-Aldrich, Ireland). Nucleic acids were re-suspended in 100 µl diethylpyrocarbonate (DEPC) treated water. DNA quality was assessed by gel electrophoresis and the Nanodrop 2000 spectrophotometer (Thermo Fisher, Ireland). DNA was quantified using the Qubit fluorometer and qubit dsDNA BR Assay Kit (Thermo Fisher, Ireland). Each sample was tested for inhibitors prior to running qPCR assays using the plasmid spike test as described in Duff et al. (2022). A qPCR was carried out to detect the presence of the inoculated bacteria (PsJN and P5) on the soaked seeds and to quantify persistence of the strains in the soil over time (Table S1). Standard curves for qPCR were constructed from the target genes. A one-in-ten dilution series of appropriate standard was used over a six-point dynamic range from 10^7 to 10^2 gene copies per µl (Table S1). Each 10 µl qPCR reaction mixture contained 5 µl SsoAdvanced Universal Probes Supermix (Biorad, USA) or Takyon™ Low ROX SYBR 2X MasterMix blue dTTP (Eurogentec, Belgium) for the PsJN probe based and P5 SYBR assays respectively, 2 µl template DNA (1 ng/µl) and primers (10 µM each of forward and reverse primers) using thermal cycling conditions specific for each assay (Table S1). No-template controls (NTC) were included in triplicates. Specificity of the amplicons for the *Bacillus sp.* P5 specific assay was confirmed by melt curve analysis at the end of each qPCR experimental run.

To assess the impact of the bioinoculant treatments on the wider soil microbiome, DNA was extracted from soil and root samples collected all plots on three sampling occasions (April 2021, October 2021 and June 2022). Prior to DNA extraction using the Dneasy PowerSoil Pro kit (Qiagen) procedure, root samples were cut into smaller fragments with a scissors (rinsing with 70% ethanol between samples) and blended using a handheld blender for 30 s. Furthermore, on plots that received a bacterial inoculant, the abundance of the microbial targets was quantified by qPCR to assess the persistence of the bioinoculants in soil. Amplicon sequencing was carried out using the primers 515F_Y/926R for 16 S rRNA gene (targeting both bacterial and archaeal communities) and ITS86F/ITS4R targeting the fungal ITS region on samples from a selection of replicates (4 per treatment group) at the three sampling times (Table S1). Sequencing of the prokaryotic and fungal communities were carried out at the Novogene Bioinformatics Technology Co., Ltd. After library preparation, sequencing was achieved using the Illumina NovaSeq platform (PE250). All the sequence data were submitted to the Sequence Read Archive National Center for Biotechnology Information database under the project accession number PRJNA1079651.

Statistical and bioinformatic analyses

All non-sequencing data was tested for normality using the Shapiro-Wilk test and log-transformed where the normality assumption was violated. Where the data met the assumptions of analysis of variance (ANOVA), the differences in the means of forage dry matter (DM) yield, and forage quality analyses between treatments were tested using a one-way ANOVA followed by a Tukey post hoc test.

All sequencing statistical analyses were performed using the R software (v. 4.0.2, R Core Team 2020). The DADA2 pipeline was used to filter, trim, merge paired end reads, remove chimeras, and construct an amplicon sequence variants (ASV) table (Callahan et al. 2016). Taxonomy was assigned using the Silva v138 database for 16 S rRNA gene reads and the UNITE v2020 database for ITS reads. Alpha diversity was calculated using 'phyloseq v 1.16.2' package (McMurdie and Holmes 2013) before the removal of singletons. The data was checked for normality using histograms and the Shapiro-Wilk's test followed by an ANOVA to check if there was a significant difference across treatments (stats v 3.6.2). Singletons were then removed and the ratios between ASVs were explored rather than relative abundance to avoid the biases associated with the compositional nature of the sequencing data (Gloor et al. 2017). The centered log-ratio (clr) transformation was applied to the ASV table with the 'compositions' package (v. 1.2.0; (van den Boogaart et al. 2018). The clr-transformed dataset was used to prepare a Principal Component Analysis (PCA) using the rda function in 'vegan' and plotted with 'ggplot' (Wickham 2009). To understand the change in community structure across treatments, a permutational multivariate analysis of variance (PERMANOVA) was performed with the adonis function in 'vegan' package using Euclidian distance (Oksanen et al. 2013). If the PERMANOVA was significant, the pairwise.adonis function in 'vegan' was used to pinpoint the significant differences between treatments.

Results

Sward yield and nutrient uptake

A visual check of the realised botanic composition of the swards showed that the plant species established in their sown sward types (Fig. S1). Sward type had a much stronger effect on forage yield than microbial inoculation in both years (Fig. 1). There was no interaction between the sward types and the inoculants in either year (Table 2). In 2021, yield in grass + legume + herb swards were higher ($P < 0.05$) than those in either grass + legume or grass-only plots (Fig. 1A). For instance, in the control plots, yield in grass + legume + herb was 3116 and 1109 kg/ha higher than in the grass-only and grass + legume, respectively. There was no effect of the microbial biofertilizer treatment on the yield values in 2021. In 2022, both grass + legume and grass + legume + herb swards had similar yields which were each significantly higher than the yield from the grass-only swards, and there was no effect of the bioinoculant treatment in these sward types (Fig. 1B). In the grass-only sward, the yield of the AMF + P5 treatment was higher than the yields of grass-only swards with all other microbial inoculant treatments and was ~46% higher than the control. Overall, the best-performing microbial inoculant treatment only increased total yield by 597 kg/ha/year, while switching from grass-only to

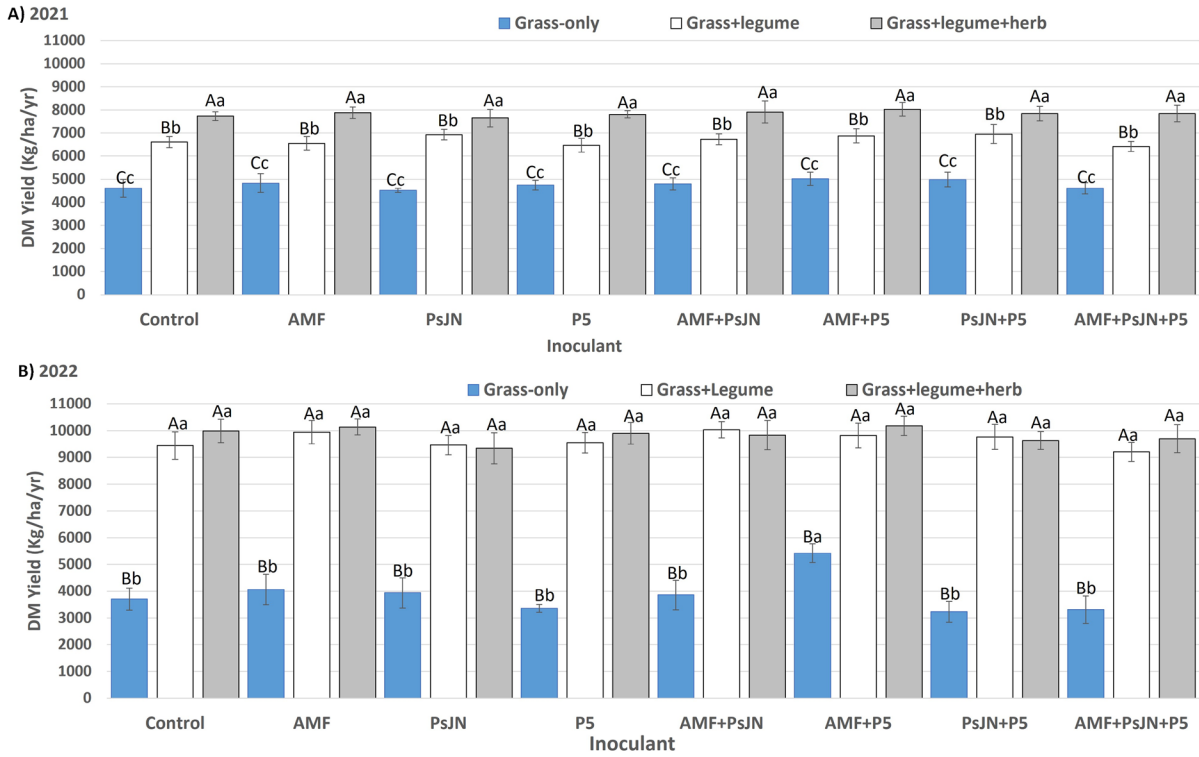


Fig. 1 Mean annual yield (±s.e.) for years 2021 and 2022. Bars with different uppercase letters denote significant differences between the sward types while bars with different lowercase letters denote significant differences between the bioinoculant types

one of the other sward types increased total yield by 3932 kg/ha/year (grass + legume) and 4693 kg/ha/year (grass + legume + herb).

Sward type had a much stronger effect on nutrient uptake than microbial inoculation in both years (Table 2). Mirroring the large effects of sward type on yield, there were very large effects of sward type on nutrient uptake; for example, nutrient uptake in grass + legume and grass + legume + herb was typically three to four times that in grass-only. There was no interaction between the sward types and the inoculants in either year (Table 2). In general, there was no effect of microbial inoculant on nutrient uptake, apart from a few exceptions (Table 3). In 2021, the grass-only swards had higher uptake in the AMF + P5 treatment for Cu (15.4%) than in the control. A higher uptake of nutrients was detected in the grass-only swards in 2022 (Table 3). Compared to the control, the AMF + P5 treatment had significantly higher values for Ca (52%), N (45%), P (56%), Mg (51%), Cu (53%) and Zn (47%) (Table 3).

Microbial biofertilizer detection and persistence

Results of qPCR assays showed that the intended bioinoculants successfully established in the seeds and in the soil + root samples, and the specific inoculated species were only detected in the treatments that they were intended to be included in (Fig. 2). There were significantly higher copies of P5 in the grass-only seeds compared to the other seeds. Bioinoculants were detected one year after application, although the copy numbers were orders of magnitude lower over time (cf. April and October 2021).

Prokaryotic and fungal community diversity and composition

Sequencing of the prokaryotic 16 S rRNA gene and the fungal ITS region were conducted to understand the diversity and composition of the microbial community and how it was impacted by microbial biofertilizer or forage sward type. Two α-diversity indices

Table 2 Effect of inoculant, sward and their interaction on dry matter yield and nutrient uptake

		2021		2022	
Parameter	Factor	F-value	P-Value	F-value	P-value
Dry matter	Inoculant	0.56	0.79	1.86	0.08
	Sward	215.57	< 0.01	475.57	< 0.01
	Inoculant*Sward	0.22	0.99	0.75	0.92
Calcium	Inoculant	0.47	0.86	2.14	0.05
	Sward	570.59	< 0.01	747.82	< 0.01
	Inoculant*Sward	0.25	0.99	0.48	0.94
Nitrogen	Inoculant	0.44	0.88	1.01	0.81
	Sward	726.09	< 0.01	464.46	< 0.01
	Inoculant*Sward	0.31	0.99	0.50	0.93
Phosphorus	Inoculant	0.48	0.85	1.58	0.15
	Sward	361.67	< 0.01	422.90	< 0.01
	Inoculant*Sward	0.18	0.99	0.61	0.86
Sulphur	Inoculant	0.14	0.99	1.23	0.29
	Sward	75.67	< 0.01	183.60	< 0.01
	Inoculant*Sward	0.15	0.99	0.39	0.98
Potassium	Inoculant	0.38	0.91	0.67	0.70
	Sward	130.00	< 0.01	113.69	< 0.01
	Inoculant*Sward	0.21	0.99	0.29	0.99
Magnesium	Inoculant	0.35	0.93	1.93	0.07
	Sward	619.70	< 0.01	463.84	< 0.01
	Inoculant*Sward	0.47	0.95	0.54	0.91
Copper	Inoculant	2.07	0.05	2.35	0.03
	Sward	112.06	< 0.01	625.12	< 0.01
	Inoculant*Sward	1.70	0.06	0.70	0.77
Zinc	Inoculant	0.72	0.65	2.20	0.04
	Sward	159.51	< 0.01	697.30	< 0.01
	Inoculant*Sward	0.82	0.65	0.83	0.64

(Chao1 and Shannon) were estimated for all treatments across sward types. No interaction between the sward types and inoculants was observed in any of the sampling times except for the fungal community Shannon index in October 2021 and June 2022. Overall, there were no differences in α-diversity between microbial treatments and sward types for the prokaryotic community (Figs. S2-S4). The only exception was in October 2021 where PsJN treated samples had a lower Chao1 index compared to the control (Fig. S3). For the fungal α-diversity, there was a significant effect of microbial treatment on the Chao1 index (April and October 2021; Figs. S5A and S6A) and the Shannon Index (October 2021; Fig. S6B). Except for April 2021 where the sward type had a significant effect on Chao1 and Shannon index (grass + legume + herb lower than both grass + legume and grass-only plots), there were no significant effect of sward type on fungal α-diversity. In general, each sward type had a high number of unique ASVs (Fig. S8).

To assess any changes in prokaryotic and fungal community structure across microbial treatments and sward types, the centred log ratio (clr) transformation was applied to the ASV tables and used to compute the PCAs. For April 2021, there were significant differences between microbial treatments (but not the sward types) for the prokaryotic community (PERMANOVA; $P > 0.05$, $R^2 = 0.10$; Fig. 3A) while for the fungal community, a significant effect of both microbial treatment (PERMANOVA; $P < 0.01$, $R^2 = 0.14$) and sward type (PERMANOVA; $P < 0.01$, $R^2 = 0.03$) was observed (Fig. 4A). However, post hoc pairwise comparison

Table 3 Nutrient uptake ($n = 6$) of harvested forage

	Grass-only	Grass + legume	Grass + legume + herb	Grass-only	Grass + legume	Grass + legume + herb
	Ca (kg/harvested dry matter/year) _2021			Ca (kg/harvested dry matter/year) _2022		
Control	28.3 ^{A}	78.1 ^{B}	99.0 ^{C}	33.2 ^{A}	120.6 ^{B}	136.2 ^{C}
AMF	27.4 ^{A}	77.7 ^{B}	103.6 ^{C}	41.2 ^{A}	117.8 ^{B}	138.4 ^{C}
PsJN	27.4 ^{A}	81.3 ^{B}	98.0 ^{C}	33.1 ^{A}	115.4 ^{B}	126.5 ^{C}
P5	28.8 ^{A}	76.6 ^{B}	101.8 ^{C}	33.3 ^{A}	110.8 ^{B}	132.5 ^{C}
AMF + PsJN	29.8 ^{A}	76.7 ^{B}	101.4 ^{C}	35.6 ^{A}	117.3 ^{B}	133.7 ^{C}
AMF + P5	29.8 ^{A}	84.3 ^{B}	102.7 ^{C}	**50.5*** ^{A}	121.1 ^{B}	133.0 ^{C}
PsJN + P5	30.4 ^{A}	84.0 ^{B}	105.3 ^{C}	30.3 ^{A}	111.1 ^{B}	130.6 ^{C}
AMF + PsJN + P5	27.6 ^{A}	76.2 ^{B}	103.3 ^{C}	30.4 ^{A}	111.1 ^{B}	122.6 ^{C}
	N (kg/harvested dry matter/year) _2021			N (kg/harvested dry matter/year) _2022		
Control	68.6 ^{A}	168.1 ^{B}	193.2 ^{C}	80.2 ^{A}	275.0 ^{B}	285.9 ^{B}
AMF	70.6 ^{A}	169.6 ^{B}	201.1 ^{C}	91.1 ^{A}	276.5 ^{B}	293.2 ^{B}
PsJN	70.1 ^{A}	176.2 ^{B}	187.6 ^{C}	82.8 ^{A}	278.3 ^{B}	260.5 ^{B}
P5	70.1 ^{A}	165.8 ^{B}	197.7 ^{C}	71.8 ^{A}	280.0 ^{B}	277.9 ^{B}
AMF + PsJN	69.5 ^{A}	173.4 ^{B}	192.1 ^{C}	81.3 ^{A}	290.7 ^{B}	272.2 ^{B}
AMF + P5	72.7 ^{A}	178.2 ^{B}	195.4 ^{C}	**116.0*** ^{A}	284.0 ^{B}	278.2 ^{B}
PsJN + P5	72.2 ^{A}	179.6 ^{B}	193.8 ^{C}	67.2 ^{A}	270.3 ^{B}	265.9 ^{B}
AMF + PsJN + P5	66.7 ^{A}	167.3 ^{B}	189.2 ^{C}	75.2 ^{A}	264.4 ^{B}	270.4 ^{B}
	P (kg/harvested dry matter/year) _2021			P (kg/harvested dry matter/year) _2022		
Control	13.8 ^{A}	22.4 ^{B}	27.0 ^{C}	12.6 ^{A}	37.6 ^{B}	39.9 ^{B}
AMF	14.2 ^{A}	22.7 ^{B}	27.2 ^{C}	14.7 ^{A}	38.1 ^{B}	40.3 ^{B}
PsJN	14.1 ^{A}	23.3 ^{B}	27.3 ^{C}	13.4 ^{A}	36.5 ^{B}	36.2 ^{B}
P5	14.7 ^{A}	21.8 ^{B}	26.1 ^{C}	10.7 ^{A}	37.2 ^{B}	38.2 ^{B}
AMF + PsJN	14.6 ^{A}	22.6 ^{B}	26.9 ^{C}	12.4 ^{A}	38.9 ^{B}	37.6 ^{B}
AMF + P5	15.1 ^{A}	23.1 ^{B}	27.8 ^{C}	**19.6*** ^{A}	38.3 ^{B}	39.1 ^{B}
PsJN + P5	14.5 ^{A}	22.3 ^{B}	26.5 ^{C}	10.7 ^{A}	37.3 ^{B}	37.5 ^{B}
AMF + PsJN + P5	13.7 ^{A}	22.1 ^{B}	26.8 ^{C}	10.9 ^{A}	36.3 ^{B}	36.3 ^{B}
	S (kg/harvested dry matter/year) _2021			S (kg/harvested dry matter/year) _2022		
Control	10.5 ^{A}	13.5 ^{B}	16.7 ^{C}	8.0 ^{A}	19.7 ^{B}	22.1 ^{B}
AMF	10.5 ^{A}	13.4 ^{B}	16.6 ^{C}	9.6 ^{A}	20.9 ^{B}	22.7 ^{B}
PsJN	10.1 ^{A}	13.6 ^{B}	16.1 ^{C}	9.1 ^{A}	19.0 ^{B}	21.0 ^{B}
P5	10.4 ^{A}	13.3 ^{B}	15.8 ^{C}	6.3 ^{A}	19.7 ^{B}	20.9 ^{B}
AMF + PsJN	10.9 ^{A}	13.4 ^{B}	15.4 ^{C}	7.5 ^{A}	20.3 ^{B}	20.2 ^{B}
AMF + P5	10.8 ^{A}	14.0 ^{B}	16.1 ^{C}	12.2 ^{A}	20.8 ^{B}	21.2 ^{B}
PsJN + P5	10.9 ^{A}	13.8 ^{B}	15.7 ^{C}	6.7 ^{A}	20.4 ^{B}	20.5 ^{B}
AMF + PsJN + P5	10.2 ^{A}	13.4 ^{B}	15.9 ^{C}	6.7 ^{A}	18.0 ^{B}	19.7 ^{B}
	K (kg/harvested dry matter/year) _2021			K (kg/harvested dry matter/year) _2022		
Control	89.5 ^{A}	147.2 ^{B}	190.6 ^{C}	65.7 ^{A}	178.7 ^{B}	191.8 ^{B}
AMF	90.1 ^{A}	152.8 ^{B}	185.7 ^{C}	74.4 ^{A}	177.3 ^{B}	190.6 ^{B}
PsJN	84.2 ^{A}	159.5 ^{B}	170.3 ^{C}	66.8 ^{A}	171.4 ^{B}	164.0 ^{B}
P5	89.8 ^{A}	147.7 ^{B}	184.3 ^{C}	55.2 ^{A}	175.3 ^{B}	191.8 ^{B}
AMF + PsJN	89.3 ^{A}	155.8 ^{B}	182.0 ^{C}	59.4 ^{A}	178.4 ^{B}	182.1 ^{B}
AMF + P5	92.8 ^{A}	155.8 ^{B}	196.7 ^{C}	88.8 ^{A}	170.9 ^{B}	190.7 ^{B}
PsJN + P5	98.6 ^{A}	162.9 ^{B}	188.2 ^{C}	55.9 ^{A}	176.5 ^{B}	171.1 ^{B}
AMF + PsJN + P5	88.4 ^{A}	147.2 ^{B}	180.5 ^{C}	52.6 ^{A}	154.9 ^{B}	162.3 ^{B}

Table 3 (continued)

	Grass-only	Grass + legume	Grass + legume + herb	Grass-only	Grass + legume	Grass + legume + herb
	Mg (kg/harvested dry matter/year) _2021			Mg (kg/harvested dry matter/year) _2022		
Control	7.5 ^{A}	17.0 ^{B}	21.0 ^{C}	7.8 ^{A}	27.3 ^{B}	28.7 ^{C}
AMF	7.4 ^{A}	17.1 ^{B}	22.3 ^{C}	9.7 ^{A}	27.1 ^{B}	30.4 ^{C}
PsJN	7.6 ^{A}	17.1 ^{B}	21.6 ^{C}	8.1 ^{A}	26.5 ^{B}	26.6 ^{C}
P5	8.3 ^{A}	16.5 ^{B}	20.6 ^{C}	7.2 ^{A}	25.4 ^{B}	27.4 ^{C}
AMF + PsJN	8.4 ^{A}	16.5 ^{B}	22.1 ^{C}	8.0 ^{A}	26.8 ^{B}	27.4 ^{C}
AMF + P5	8.4 ^{A}	16.5 ^{B}	22.2 ^{C}	**11.8*** ^{A}	25.9 ^{B}	28.4 ^{C}
PsJN + P5	7.9 ^{A}	17.1 ^{B}	21.9 ^{C}	6.6 ^{A}	24.9 ^{B}	27.3 ^{C}
AMF + PsJN + P5	7.1 ^{A}	15.8 ^{B}	22.1 ^{C}	6.4 ^{A}	24.8 ^{B}	25.6 ^{C}
	Cu (g/harvested dry matter/year) _2021			Cu (g/harvested dry matter/year) _2022		
Control	22.7 ^{A}	45.6 ^{B}	66.2 ^{C}	23.4 ^{A}	81.0 ^{B}	88.6 ^{C}
AMF	22.8 ^{A}	45.7 ^{B}	64.4 ^{C}	28.0 ^{A}	77.7 ^{B}	91.7 ^{C}
PsJN	25.4 ^{A}	49.7 ^{B}	100.0 ^{C}	23.3 ^{A}	81.9 ^{B}	80.5 ^{C}
P5	25.8 ^{A}	46.4 ^{B}	63.7 ^{C}	23.2 ^{A}	79.4 ^{B}	83.6 ^{C}
AMF + PsJN	25.5 ^{A}	49.9 ^{B}	61.3 ^{C}	24.5 ^{A}	84.8 ^{B}	87.0 ^{C}
AMF + P5	**26.2*** ^{A}	51.7 ^{B}	64.2 ^{C}	**35.9*** ^{A}	84.5 ^{B}	87.9 ^{C}
PsJN + P5	**28.5*** ^{A}	49.2 ^{B}	63.7 ^{C}	21.8 ^{A}	75.7 ^{B}	84.3 ^{C}
AMF + PsJN + P5	21.8 ^{A}	47.7 ^{B}	59.9 ^{C}	20.4 ^{A}	74.1 ^{B}	77.8 ^{C}
	Zn (g/harvested dry matter/year) _2021			Zn (g/harvested dry matter/year) _2022		
Control	106.4 ^{A}	179.0 ^{B}	251.9 ^{C}	73.7 ^{A}	233.8 ^{B}	296.8 ^{C}
AMF	105.6 ^{A}	176.1 ^{B}	249.5 ^{C}	89.2 ^{A}	229.3 ^{B}	304.8 ^{C}
PsJN	111.9 ^{A}	176.3 ^{B}	265.3 ^{C}	73.9 ^{A}	236.6 ^{B}	285.6 ^{C}
P5	137.0 ^{A}	189.8 ^{B}	222.7 ^{C}	71.2 ^{A}	244.1 ^{B}	287.7 ^{C}
AMF + PsJN	133.9 ^{A}	172.6 ^{B}	250.4 ^{C}	75.8 ^{A}	259.9 ^{B}	314.2 ^{C}
AMF + P5	128.5 ^{A}	163.4 ^{B}	251.1 ^{C}	**108.4*** ^{A}	238.0 ^{B}	315.7 ^{C}
PsJN + P5	113.5 ^{A}	170.4 ^{B}	235.2 ^{C}	71.5 ^{A}	220.2 ^{B}	297.8 ^{C}
AMF + PsJN + P5	92.5 ^{A}	167.7 ^{B}	232.4 ^{C}	69.6 ^{A}	227.3 ^{B}	255.4 ^{C}

Numbers in bold with * indicate significant differences with the control. Different letters within the same row for the same element within the same year indicate significant ($P < 0.05$) differences between the swards

between both microbial treatments and sward types with Benjamini Hochberg correction for multiple comparisons showed no significant differences. Due to correction for multiple testing, post hoc tests using pairwise Adonis is stricter than PERMANOVA. For October 2021, both microbial treatments and sward type had a significant effect on both the prokaryotic (PERMANOVA; $P < 0.01$, $R^2 = 0.26$; Fig. 3B) and fungal (PERMANOVA; $P < 0.01$, $R^2 = 0.26$; Fig. 4B) community structures, respectively. However, similar to April 2021, pairwise *post hoc* test did not reveal any significant differences. Similarly, for June 2022, there was a significant effect of both microbial treatment and sward type on both the prokaryotic (PERMANOVA; $P < 0.05$, $R^2 = 0.10$; Fig. 3C) and fungal (PERMANOVA; $P < 0.01$, $R^2 = 0.20$; Fig. 4C) community structures. No significant differences were detected by the pairwise comparisons.

Discussion

Bioinoculants persisted in soil, with little or no effects on yield and nutrient uptake

For the most part, we found no effect of the bioinoculant treatments on yield or nutrient uptake, and this was consistent across the three sward types. However, the AMF + P5 treatment increased forage yield in the

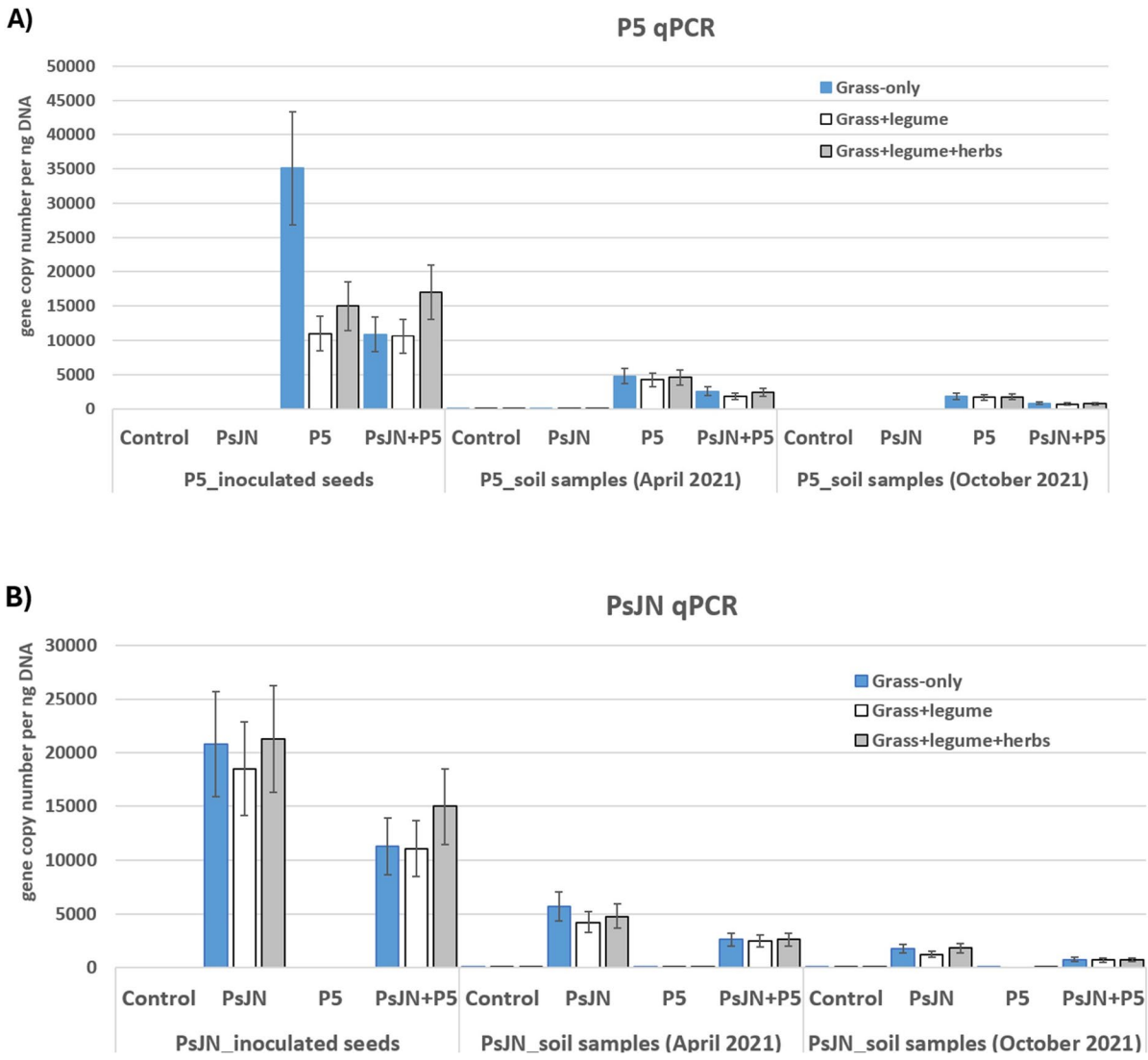


Fig. 2 qPCR result in the inoculated seeds and field samples post application for the (**A**) P5 and (**B**) PsJN bacterial strains ($n = 6$)

second year in the grass-only sward, although this yield was still far lower than that of the clover-containing swards. This effect of AMF + P5 could be due to synergistic interactions between the bacteria and the AMF resulting in a higher plant growth promotion than either of them applied singly. Further, some of the AMF containing treatments increased uptake of selected nutrients (Ca, N, P and S) compared to the control. The bacterial bioinoculants could be detected for at least a year after application. This provides some evidence that they were capable of occupying niche space within the rhizosphere or endosphere. Although we did not assess the persistence of the AMF directly, the effects of some selected AMF treatments indicate the successful application of the mycorrhizal treatments. Overall, the experimental protocols resulted in successful inoculation, persistence of the bacterial strains for at least one year, and no evidence of cross-contamination of the bioinoculant treatments.

The persistence of microbial inoculants in soil is a critical aspect influencing their effectiveness and subsequent adoption in sustainable agriculture. However, persistence does not necessarily equate to

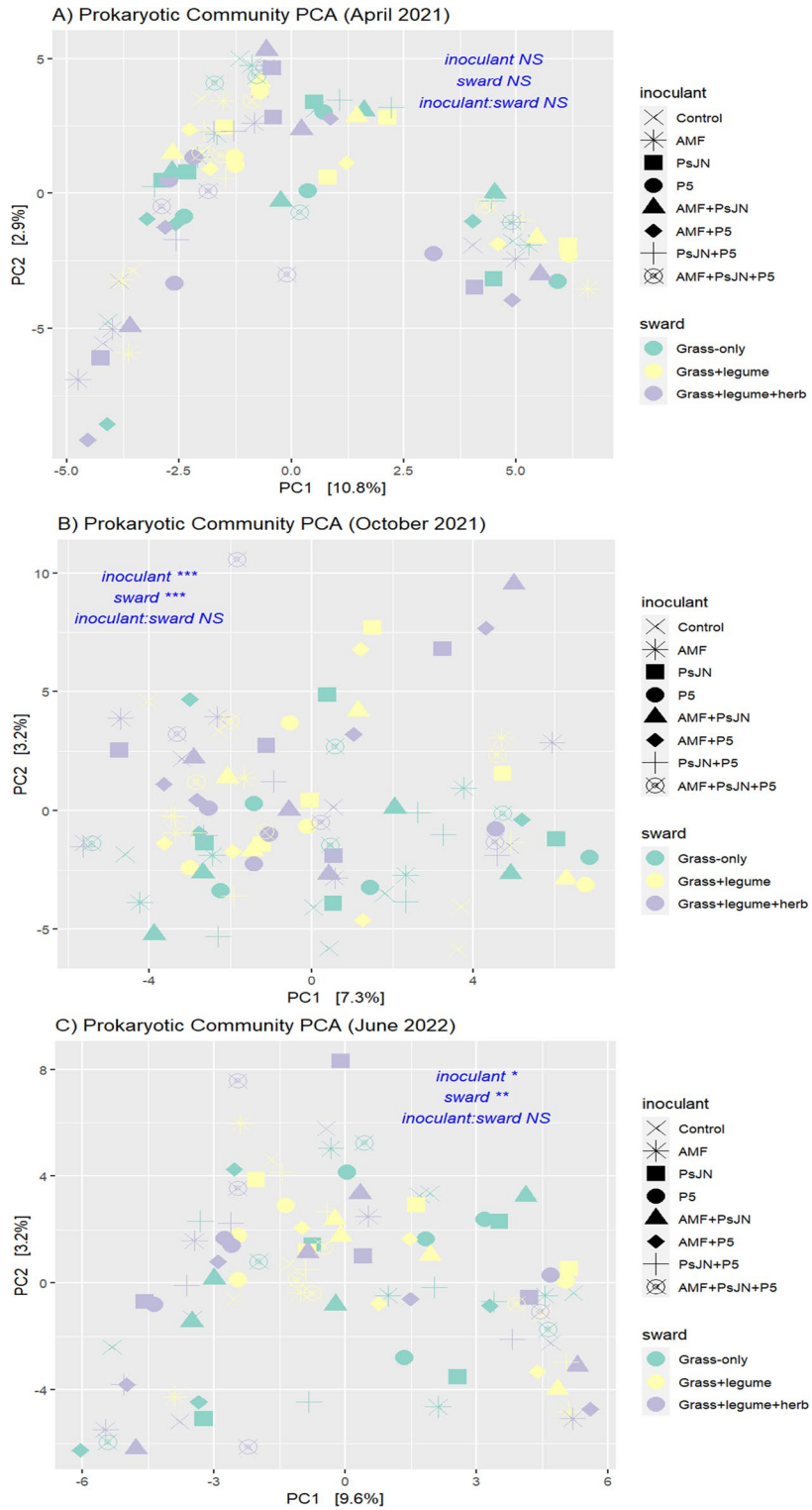


Fig. 3 Principal Component Analysis (PCA) based on centred log ratio (clr) transformed ASV data of prokaryotic community for (**A**) April 2021, (**B**) October 2021, and (**C**) June 2022. Blue text within each panel indicates the effect of treatments as: *= $P<0.05$, **= $P<0.01$, ***= $P<0.001$, NS = not significant

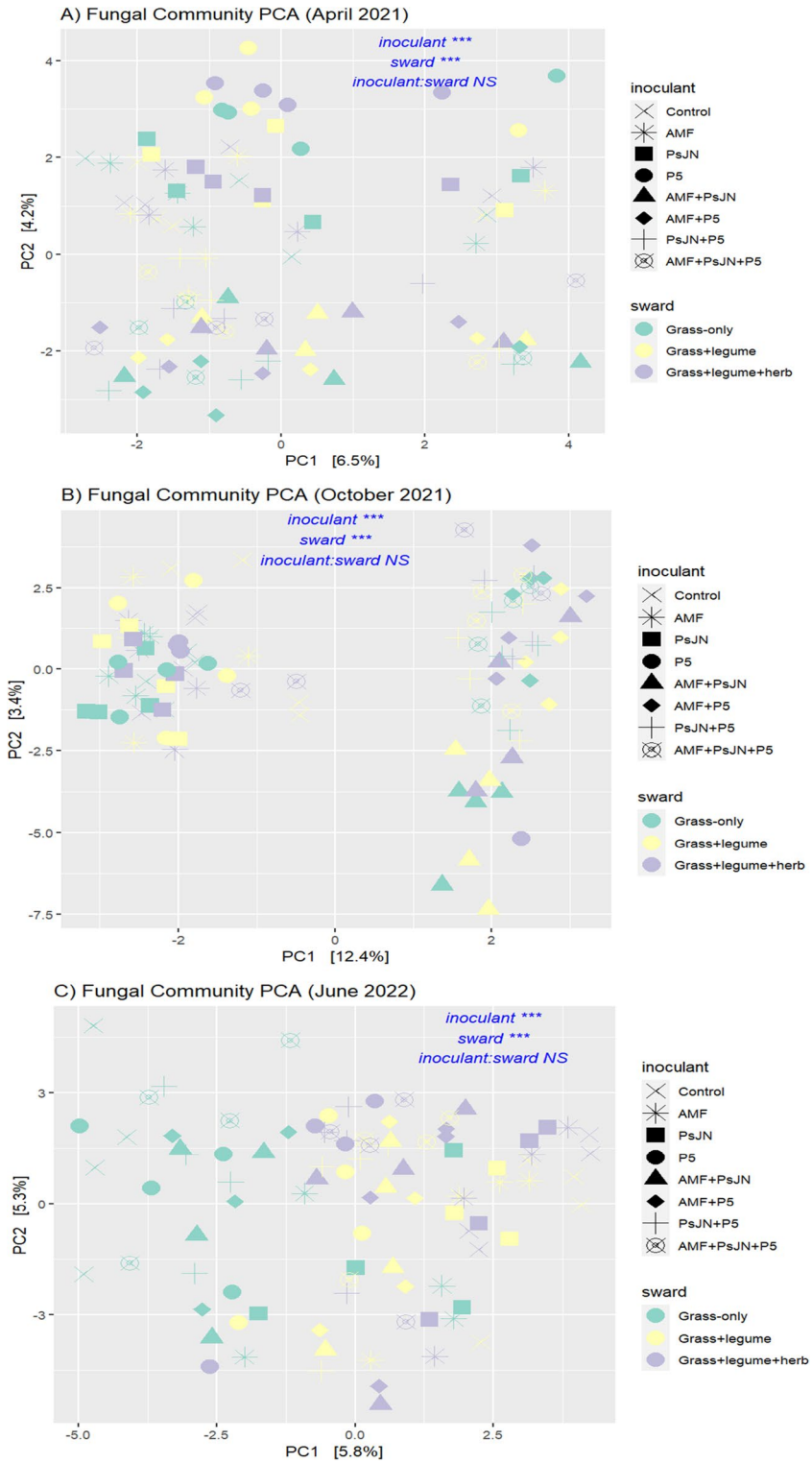


Fig. 4 Principal Component Analysis (PCA) based on centred log ratio (clr) transformed ASV data of fungal community for (**A**) April 2021, (**B**) October 2021, and (**C**) June 2022. Blue text within each panel indicates the effect of treatments as: *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$, NS = not significant

effectiveness, and it may be the case that any benefits associated with bioinoculants may manifest only in soils that are somewhat degraded or where the plant is undergoing physiological stress, e.g., due to nutrient limitation or drought conditions (Benmrid et al. 2023; Naveed et al. 2014; O'Callaghan et al. 2022; Sheibani-Tezerji et al. 2015).

In this study, the lower than the agronomically recommended levels of N applied to *L. perenne* swards, and the resulting low yields strongly indicate that it experienced nitrogen limitation (at least). Even in this context, the effectiveness of the bioinoculants was modest. The persistence and effectiveness of microbial inoculants can vary based on factors that include soil type, nutrient availability, moisture availability, the nature of the indigenous microbial community, host plant identity, plant growth stage, the physiological stress of the plant (abiotic and biotic), and the specific requirements of the applied microorganisms (Cornell et al. 2021; Jing et al. 2017; Mander et al. 2012; Verbruggen et al. 2013); this makes predictions of the outcomes of bioinoculant addition challenging. Bashan and Holguin (1998) stressed the importance of considering soil types when assessing the persistence of nitrogen-fixing bacteria. Sharma et al. (2013) highlighted the need to understand the survival and activity of phosphate-solubilizing bacteria over time. In the present study, applied bioinoculants were detected one year after application (although in amounts that were several orders of magnitude lower than at the time of application). There is a need for further research to determine how the persistence and function of microbial biofertilizers can be improved under different soil and environmental conditions, to investigate their sustained positive impact on soil health and plant growth. A plausible research pathway would be to also test whether these bioinoculants can be applied in combination with varying amounts of chemical fertilizer, to directly test their fertilizer replacement value, and any antagonistic or synergistic interactions. Similarly, investigations could incorporate experimentally controlled gradients of stress to test the effectiveness of bioinoculants (Cornell et al. 2021; O'Callaghan et al. 2022).

Strong effects of sward type on yield and total nutrient uptake

In an intensively managed temperate grassland, we conducted a novel field experiment that assessed the impact of microbial inoculants and sward type on yield, nutrient uptake, and the wider soil microbial communities. Sward type had a much stronger effect on forage yield and nutrient uptake compared to the microbial inoculants. Specifically, significantly higher forage yield and nutrient uptake were obtained in the more diverse swards (grass + legume and grass + legume + herb) compared to the monoculture (grass-only). Several studies have shown the positive effects of clover-containing grass communities on yield (Baker et al. 2023; Dewhurst et al. 2009; Finn et al. 2013; Grace et al. 2018; Grange et al. 2021 scher et al. 2022), which is strongly related to the clover content and the degree of symbiotic nitrogen fixation. As the agronomically applied amount of nitrogen declines, the relative contribution of the symbiotically fixed N becomes more pronounced both in absolute terms (Nyfeler et al. 2011) and relative to the grass-only community (Grange et al. 2021; Nyfeler et al. 2009, 2011). For instance, Nyfeler et al. (2009) showed that the yield of grass-clover communities with ~30–70% clover and receiving 50 kg N could outyield grass-only communities receiving 450 kg N. The degree of contribution of symbiotically fixed N and other synergistic plant interactions can be sufficiently strong for clover-containing communities to yield more than the best-performing monoculture (Finn et al. 2013; Grange et al. 2021; Lüscher et al. 2022; Nyfeler et al. 2009). In this study, there was a very strong difference (on average 3900 and 4700 kg/ha/year for grass + legume and grass + legume + herb, respectively) in yield between the grass-only and legume (clover-containing) communities, reflecting low application of nitrogen. This low application was a design feature of the experiment to give a better opportunity for any positive effects of the bioinoculants to be expressed; at higher levels of N and P input, such effects may not be apparent to the same extent.

Native microbial community minimally affected by sward type and bioinoculants

Understanding the effects of the bioinoculant on the native communities is critically important for the wider implementation of this technology (Cornell et al. 2021), including their capacity to: interact with the community members, become invasive, or impact community function. In the present study, both the bioinoculants and sward type affected the prokaryotic and fungal community composition (PERMANOVA); however, after correction for multiple comparisons in *post hoc* analysis, differences were insufficiently large to be detected in pairwise comparisons. The reported effect of microbial inoculants on the indigenous microbial community is inconsistent and can vary from significant to no effects (Cornell et al. 2021; Trabelsi and Mhamdi 2013). Furthermore, the impact of microbial inoculants is subject to strong temporal variation; both in terms of the dynamic nature of the resident community into which the bioinoculant is inserted, which itself adapts continually in response to dynamic prevailing local conditions (Köberl et al. 2020), and the success and population dynamics of the bioinoculant itself, which in some cases may establish only transiently or struggle to compete with the resident soil microbiome (Compant et al. 2019; Pereg et al. 2014). More context-specific studies are essential to understanding the extent of the effect of microbial inoculant on different soils and ecosystems (Schmidt and Gaudin 2018). The impact of diversifying plant communities in intensively managed grassland swards on microbial communities has received limited investigation to date. Ryan et al. (2023) found that plant composition impacted prokaryotic communities at in the top 15 cm of bulk soil and fungal communities between 15 and 30 cm in depth. Ikoyi et al. (2023) showed that multispecies swards was associated with an altered soil nematode community structure and changes in relative abundance of nematode functional groups, indicating impacts on the wider food web and predators that modulate microbial communities.

Investigating the use of microbial inoculants: extrapolating from laboratory to field

The use of microbial inoculants has been promoted as a sustainable agricultural practice to support long-term soil fertility for crop productivity, with lower environmental impact. However, most studies that show benefits from the application of bioinoculants have been critiqued for limitations associated with their short term duration, limited spatial scale and strong edge effects in pots and other mesocosms, removal of the native microbial community by sterilisation, and using controlled plant growth conditions that limit the range of environmental variation experienced by the plants and microorganisms (Meyer et al. 2019; O' Callaghan et al. 2022). The key question here is whether these benefits observed in very controlled and artificial conditions can be obtained in field conditions. There has been a dearth of research on the field-scale investigation of microbial inoculants in grassland systems, and a corresponding combination with varying sward diversity in grassland systems, at the field scale. Such knowledge is essential if policy objectives to reduce N fertiliser use are to be implemented at farm-scale following advice, decision-making and adoption by farmers. This knowledge needs to be relevant at farm-scale and will require further field-scale testing for grassland communities.

Our field experiment was conducted under more controlled conditions, and a real-world application in grassland in a commercial farm setting would have additional challenges for effects of bioinoculants to be realised: infrequent reseeding; the inability to compete with native microbiome where a healthy biological community is established; complicating management factors such as application of fertiliser; regular defoliation of the plants; hotspots of urine and dung, etc.

Conclusions

This is one of the first studies to test the impact of microbial biofertilizers in different sward types under field conditions in an intensively managed grassland scenario. Under the tested experimental conditions, the bioinoculants successfully colonized the sward types and persisted for at least one year. There was little or no effect of the bioinoculants on forage yield and nutrient uptake, and this was consistent across three forage sward types that differed in diversity of agronomic plant species. The combined application of rhizosphere bacteria (*Bacillus* sp. P5)

and arbuscular mycorrhizal fungi (AMF) showed some potential to increase forage yield in perennial ryegrass under nitrogen limitation, and modestly increased nutrient uptake of selected nutrients. There was a modest effect of sward type and bioinoculant on the native microbial community. Overall, the effect of sward type on forage yield and nutrient uptake was more significant, and there was little to no effect of the application of microbial inoculants across the sward types. As a practical, farm-scale management strategy to reduce fertiliser use, a focus on the incorporation of clover was far more effective than the application of microbial inoculants.

Acknowledgements We would like to thank James Reck, Rioch Fox and John Cardiff for helping with the mechanical harvesting, Technical Staff in Johnstown Castle and all those that helped with sowing the field trial. This work was funded by the EU Horizon Research and Innovation programme under the MASTER project (grant agreement No. 818368).

Author contributions Israel Ikoyi, Fiona Brennan, John Finn, and David Wall designed the study. Data collection and analyses was by Israel Ikoyi. Initial draft of the manuscript was written by Israel Ikoyi, Fiona Brennan, and John Finn. All authors provided comments, suggestions, and approved the final version of the manuscript.

Funding Open Access funding provided by the IReL Consortium. This work was funded by the EU Horizon Research and Innovation programme under the MASTER project (grant agreement No. 818368).

Data availability The prokaryotic and fungal sequences obtained in this study are available at the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/PRJNA1079651) under the project accession number PRJNA1079651.

Declarations

Competing interests Author Eva Lucic-Mercy is employed by INOQ GmbH. The remaining authors declare that they have no known competing financial interests or personal relationships that influenced the work reported in this paper.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Augé RM, Stodola AJ, Tims JE, Saxton AM (2001) Moisture retention properties of a mycorrhizal soil. Plant Soil 230:87–97. https://doi.org/10.1023/A:1004891210871

Baker S, Lynch MB, Godwin F, Boland TM, Kelly AK, Evans AC, Murphy PN, Sheridan H (2023) Multispecies swards outperform perennial ryegrass under intensive beef grazing. Agric Ecosyst Environ 345:108335. https://doi.org/10.1016/j.agee.2022.108335

Bashan Y, Holguin G (1998) Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant-growth-promoting bacteria) and PGPB. Soil Biol Biochem 30:1225–1228. https://doi.org/10.1016/S0038-0717(97)00187-9

Bauer JT, Kleczewski NM, Bever JD, Clay K, Reynolds HL (2012) Nitrogen-fixing bacteria, arbuscular mycorrhizal fungi, and the productivity and structure of prairie grassland communities. Oecologia 170:1089–1098. https://doi.org/10.1007/s00442-012-2363-3

Bell MJ, Rees RM, Cloy JM, Topp CF, Bagnall A, Chadwick DR (2015) Nitrous oxide emissions from cattle excreta applied to a Scottish grassland: effects of soil and climatic conditions and a nitrification inhibitor. Sci Total Environ 508:343–353. https://doi.org/10.1016/j.scitotenv.2014.12.008

Benmrid B, Ghoulam C, Zeroual Y, Kouisni L, Bargaz A (2023) Bioinoculants as a means of increasing crop tolerance to drought and phosphorus deficiency in legume-cereal intercropping systems. Commun Biol 6(1):1016. https://doi.org/10.1038/s42003-023-05399-5

Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front Microbiol 6:169741. https://doi.org/10.3389/fmicb.2015.01559

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13(7):581–583. https://doi.org/10.1038/nmeth.3869

Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of Earth's nitrogen cycle. Science 330(6001):192–196. https://doi.org/10.1126/science.1186120

Compant S, Samad A, Faist H, Sessitsch A (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. J Adv Res 19:29–37. https://doi.org/10.1016/j.jare.2019.03.004

Connolly J, Sebastià MT, Kirwan L, Finn JA, Llurba R, Suter M, Collins RP, Porqueddu C, Helgadóttir Á, Baadshaug OH, Bélanger G (2018) Weed suppression greatly increased by plant diversity in intensively managed grasslands: a continental-scale experiment. J Appl Ecol 55(2):852–862. https://doi.org/10.1111/1365-2664.12991

Cornell C, Kokkoris V, Richards A, Horst C, Rosa D, Bennett JA, Hart MM (2021) Do bioinoculants affect resident microbial communities? A meta-analysis. Front Agron 3:753474. https://doi.org/10.3389/fagro.2021.753474

Cummins S, Finn JA, Richards KG, Lanigan GJ, Grange G, Brophy C, Cardenas LM, Misselbrook TH, Reynolds CK, Krol DJ (2021) Beneficial effects of multi-species mixtures on N2O emissions from intensively managed grassland swards. Sci Total Environ 792:148163. https://doi.org/10.1016/j.scitotenv.2021.148163

Dewhurst RJ, Delaby L, Moloney A, Boland T, Lewis E (2009) Nutritive value of forage legumes used for grazing and silage. IJAFR 1:167–187

Divan Baldani VL, Baldani JI, Döbereiner J (2000) Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* Spp. Biol Fertil Soils 30:485–491. https://doi.org/10.1007/s003740050027

Duff AM, Forrestal P, Ikoyi I, Brennan F (2022) Assessing the long-term impact of urease and nitrification inhibitor use on microbial community composition, diversity and function in grassland soil. Soil Biol Biochem 170:108709. https://doi.org/10.1016/j.soilbio.2022.108709

Esmaeel Q, Miotto L, Rondeau M, Leclère V, Clément C, Jacquard C, Sanchez L, Barka EA (2018) *Paraburkholderia phytofirmans* PsJN-plants interaction: from perception to the induced mechanisms. Front Microbiol 9:2093. https://doi.org/10.3389/fmicb.2018.02093

Finn JA, Kirwan L, Connolly J, Sebastià MT, Helgadottir A et al (2013) Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continental-scale field experiment. J Appl Ecol 50(2):365–375. https://doi.org/10.1111/1365-2664.12041

Frame J, Laidlaw AS (2011) Improved grassland management. The Crowood Press Ltd. New edition, Marlborough

Galindo FS, da Silva EC, Pagliari PH, Fernandes GC, Rodrigues WL, Biagini AL, Baratella EB, da Silva Júnior CA, Neto MJ, Silva VM, Muraoka T, Teixeira Filho MCM (2021) Nitrogen recovery from fertilizer and use efficiency response to *Bradyrhizobium* sp. and *Azospirillum brasilense* combined with N rates in cowpea-wheat crop sequence. Appl Soil Ecol 157:103764. https://doi.org/10.1016/j.apsoil.2020.103764

Glick BR (2004) Bacterial ACC deaminase and the alleviation of plant stress. Adv Appl Microbiol 56:291–312

Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ (2017) Microbiome datasets are compositional: and this is not optional. Front Microbiol 8:2224. https://doi.org/10.3389/fmicb.2017.02224

Grace C, Boland TM, Sheridan H, Lott S, Brennan E, Fritch R, Lynch MB (2018) The effect of increasing pasture species on herbage production, chemical composition and utilization under intensive sheep grazing. Grass Forage Sci 73(4):852–864

Grange G, Finn JA, Brophy C (2021) Plant diversity enhanced yield and mitigated drought impacts in intensively managed grassland communities. J Appl Ecol 58(9):1864–1875. https://doi.org/10.1111/1365-2664.13894

Gravel V, Antoun H, Tweddell RJ (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol Biochem 39(8):1968–1977. https://doi.org/10.1016/j.soilbio.2007.02.015

Ikoyi I, Grange G, Finn JA, Brennan FP (2023) Plant diversity enhanced nematode-based soil quality indices and changed soil nematode community structure in intensively-managed agricultural grasslands. Eur J Soil Biol 118:103542. https://doi.org/10.1016/j.ejsobi.2023.103542

Issa A, Esmaeel Q, Sanchez L, Courteaux B, Guise JF, Gibon Y, Ballias P, Clément C, Jacquard C, Vaillant-Gaveau N, Aït Barka E (2018) Impacts of *Paraburkholderia phytofirmans* strain PsJN on tomato (*Lycopersicon esculentum* L.) under high temperature. Front Plant Sci 9:1397. https://doi.org/10.3389/fpls.2018.01397

Jing Z, Chen R, Wei S, Feng Y, Zhang J, Lin X (2017) Response and feedback of C mineralization to P availability driven by soil microorganisms. Soil Biol Biochem 105:111–120. https://doi.org/10.1016/j.soilbio.2016.11.014

Kim S, Lowman S, Hou G, Nowak J, Flinn B, Mei C (2012) Growth promotion and colonization of switchgrass (*Panicum virgatum*) cv. Alamo by bacterial endophyte *Burkholderia phytofirmans* strain PsJN. Biotechnol Biofuels 5(1):1–10. https://doi.org/10.1186/1754-6834-5-37

Köberl M, Wagner P, Müller H, Matzer R, Unterfrauner H, Cernava T, Berg G (2020) Unraveling the complexity of soil microbiomes in a large-scale study subjected to different agricultural management in Styria. Front Microbiol 11:1052. https://doi.org/10.3389/fmicb.2020.01052

Lekberg Y, Helgason T (2018) In situ mycorrhizal function–knowledge gaps and future directions. New Phytol 220(4):957–962. https://doi.org/10.1111/nph.15064

Li J, Wang J, Liu H, Macdonald CA, Singh BK (2022) Application of microbial inoculants significantly enhances crop productivity: a meta-analysis of studies from 2010 to 2020. JSAE 1(3):216–225. https://doi.org/10.1002/sae2.12028

Lüscher A, Barkaoui K, Finn JA, Suter D, Suter M, Volaire F (2022) Using plant diversity to reduce vulnerability and increase drought resilience of permanent and sown productive grasslands. Grass Forage Sci 77(4):235–246. https://doi.org/10.1111/gfs.12578

Lutz S, Bodenhausen N, Hess J, Valzano-Held A, Waelchli J, Deslandes-Hérold G, Schlaeppi K, van der Heijden MG (2023) Soil microbiome indicators can predict crop growth response to large-scale inoculation with arbuscular mycorrhizal fungi. Nat Microbiol 8(12):2277–2289. https://doi.org/10.1038/s41564-023-01577-7

Mander C, Wakelin S, Young S, Condron L, O'Callaghan M (2012) Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. Soil Biol Biochem 44(1):93–101. https://doi.org/10.1016/j.soilbio.2011.09.009

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102. https://doi.org/10.1007/BF00000098

McMurdie PJ, Holmes S (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8(4):e61217. https://doi.org/10.1371/journal.pone.0061217

Meyer G, Maurhofer M, Frossard E, Gamper HA, Mäder P, Mészáros É, Schönholzer-Mauclaire L, Symanczik S, Oberson A (2019) *Pseudomonas protegens* CHA0 does not increase phosphorus uptake from 33P labeled synthetic hydroxyapatite by wheat grown on calcareous soil. Soil Biol Biochem 131:217–228. https://doi.org/10.1016/j.soilbio.2019.01.015

Mitter B, Pfaffenbichler N, Flavell R, Compant S, Antonielli L, Petric A, Berninger T, Naveed M, Sheibani-Tezerji R, Von Maltzahn G, Sessitsch A (2017) A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. Front Microbiol 8:11. https://doi.org/10.3389/fmicb.2017.00011

Moloney T, Sheridan H, Grant J, O'Riordan EG, O'Kiely P (2020) Yield of binary-and multi-species swards relative to single-species swards in intensive silage systems. IJAFR 59(1):12–26. https://doi.org/10.2478/ijafr-2020-0002

Naveed M, Hussain MB, Zahir ZA, Mitter B, Sessitsch A (2014) Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. Plant Growth Regul 73:121–131. https://doi.org/10.1007/s10725-013-9874-8

Naveed M, Qureshi MA, Zahir ZA, Hussain MB, Sessitsch A, Mitter B (2015) L-Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by *Burkholderia phytofirmans* PsJN. Ann Microbiol 65:1381–1389. https://doi.org/10.1007/s13213-014-0976-y

Newsham KK, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. TREE 10(10):407–411. https://doi.org/10.1016/S0169-5347(00)89157-0

Nyfeler D, Huguenin-Elie O, Suter M, Frossard E, Connolly J, Lüscher A (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. J Appl Ecol 46(3):683–691. https://doi.org/10.1111/j.1365-2664.2009.01653.x

Nyfeler D, Huguenin-Elie O, Suter M, Frossard E, Lüscher A (2011) Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. Agric Ecosyst Environ 140(1–2):155–163. https://doi.org/10.1016/j.agee.2010.11.022

O'Callaghan M, Ballard RA, Wright D (2022) Soil microbial inoculants for sustainable agriculture: limitations and opportunities. Soil Use Manag 38(3):1340–1369. https://doi.org/10.1111/sum.12811

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MH, Wagner H, Oksanen MJ (2013) Vegan: community ecology package. R package version 2.0-7. https://CRAN.Rproject.org/package=vegan

Omirou M, Fasoula DA, Ioannides IM (2016) *Bradyrhizobium* inoculation alters indigenous AMF community assemblages and interacts positively with AMF inoculum to improve cowpea performance. Appl Soil Ecol 108:381–389. https://doi.org/10.1016/j.apsoil.2016.09.018

Pánková H, Lepinay C, Rydlová J, Voříšková A, Janoušková M, Dostálek T, Münzbergová Z (2018) Arbuscular mycorrhizal fungi and associated microbial communities from dry grassland do not improve plant growth on abandoned field soil. Oecologia 186:677–689. https://doi.org/10.1007/s00442-017-4054-6

Pereg L, Alderman J, McMillan M (2014) Scoping the potential uses of beneficial microorganisms for increasing productivity in cotton cropping systems. Soil Biol Biochem 78:77–89. https://doi.org/10.1016/j.soilbio.2014.10.020

Pillay VK, Nowak J (1997) Inoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. Can J Microbiol 43(4):354–361. https://doi.org/10.1139/m97-049

R Core Team (2020) R: a language and environment for statistical computing. Foundation for statistical computing. https://www.R-project.org/. Accessed 8 Aug 2023

Rahman M, Sabir AA, Mukta JA, Khan MM, Mohi-Ud-Din M, Miah MG, Rahman M, Islam MT (2018) Plant probiotic bacteria *bacillus* and *Paraburkholderia* improve growth, yield and content of antioxidants in strawberry fruit. Sci Rep 8(1):2504. https://doi.org/10.1038/s41598-018-20235-1

Reheul D, Cougnon M, Kayser M, Pannecoucque J, Swanckaert J, De Cauwer B, van den Pol-van Dasselaar A, De Vliegher A (2017) Sustainable intensification in the production of grass and forage crops in the low countries of north-west Europe. Grass Forage Sci 72(3):369–381. https://doi.org/10.1111/gfs.12285

Ryan KB, De Menezes A, Finn JA, Brennan FP (2023) Plant species and soil depth differentially affect microbial diversity and function in grasslands. JSAE 2(4):397–411. https://doi.org/10.1002/sae2.12077

Schmidt JE, Gaudin AC (2018) What is the agronomic potential of biofertilizers for maize? A meta-analysis. FEMS Microbiol Ecol 94(7):fiy094. https://doi.org/10.1093/femsec/fiy094

Schütz L, Gattinger A, Meier M, Müller A, Boller T, Mäder P, Mathimaran N (2018) Improving crop yield and nutrient use efficiency via biofertilization—A global meta-analysis. Front Plant Sci 8:2204. https://doi.org/10.3389/fpls.2017.02204

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2:1–14. https://doi.org/10.1186/2193-1801-2-587

Sheibani-Tezerji R, Rattei T, Sessitsch A, Trognitz F, Mitter B (2015) Transcriptome profiling of the endophyte *Burkholderia phytofirmans* PsJN indicates sensing of the plant environment and drought stress. MBio 6(5):10–1128. https://doi.org/10.1128/mbio.00621-15

Shi J, Wang X, Wang E (2023) Mycorrhizal symbiosis in plant growth and stress adaptation: from genes to ecosystems. Annu Rev Plant Biol 74:569–607. https://doi.org/10.1146/annurev-arplant-061722-090342

Shnel A, Tracy S, Schmidt O, Murphy P, Lynch MB (2021) The importance of forage legume inclusion in agricultural swards to enhance earthworm activity and water infiltration rates. The XXIV international grassland congress/XI international rangeland congress (Sustainable use of

grassland and rangeland resources for improved livelihoods). https://uknowledge.uky.edu/igc/24/4/4

Teague R, Kreuter U (2020) Managing grazing to restore soil health, ecosystem function, and ecosystem services. Front Sustain Food Syst 157:157. https://doi.org/10.3389/fsufs.2020.534187

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418(6898):671–677. https://doi.org/10.1038/nature01014

Trabelsi D, Mhamdi R (2013) Microbial inoculants and their impact on soil microbial communities: a review. BioMed Res Int 2013:863240. https://doi.org/10.1155/2013/863240

USEPA (1996) EPA Method 3052: microwave assisted acid digestion of siliceous and organically based matrices. Test methods for evaluating solid waste. USEPA, Washington, DC

van den Boogaart KG, Tolosana-Delgado R, Bren M (2018) R package 'compositions': compositional data analysis. https://CRAN.R-project.org/package=compositions

Verbruggen E, van der Heijden MG, Rillig MC, Kiers ET (2013) Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol 197(4):1104–1109. https://doi.org/10.1111/j.1469-8137.2012.04348.x

Vessey JK, Buss TJ (2002) *Bacillus cereus* UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes: controlled-environment studies. Can J Plant Sci 82(2):282–290. https://doi.org/10.4141/P01-047

Walder F, Van Der Heijden MG (2015) Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. Nat Plants 1(11):1–7. https://doi.org/10.1038/nplants.2015.159

Wang B, Mei C, Seiler JR (2015) Early growth promotion and leaf level physiology changes in *Burkholderia phytofirmans* strain PsJN inoculated switchgrass. Plant Physiol Biochem 86:16–23. https://doi.org/10.1016/j.plaphy.2014.11.008

Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer, New York

Yu L, Zhang H, Zhang W, Liu K, Liu M, Shao X (2022) Cooperation between arbuscular mycorrhizal fungi and plant growth-promoting bacteria and their effects on plant growth and soil quality. PeerJ 10:e13080. https://doi.org/10.7717/peerj.13080

Zhang Y, Gao X, Shen Z et al (2019) Pre-colonization of PGPR triggers rhizosphere microbiota succession associated with crop yield enhancement. Plant Soil 439:553–567. https://doi.org/10.1007/s11104-019-04055-4

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.