# Pasture-crop rotations modulate the soil and rhizosphere microbiota and preserve soil structure supporting oat cultivation in the Pampa biome

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## ABSTRACT

Mixed systems of grain and livestock production based on pasture-crop rotations are a promising strategy to promote agriculture resilience and allow an ecological intensification of agriculture yet little is known about underlying processes in soil. To test the hypothesis that pasture-crop rotations preserve soil structure and select for beneficial soil and rhizosphere microbiota, supporting soil health and grain production, a long-term field experiment under no-tillage was studied. The experiment evaluated a gradient of land use intensities and vegetation diversities, from highly intensive continuous cropping to the least intensive system i.e. a nearby natural grassland, with two intermediate land use intensities i.e. short pasture-crop rotation and long pasture-crop rotation. Soil health was assessed based on soil physicochemical properties, microbial (Bacteria/Archaea and Fungi) community diversity and composition and oat performance. Pasture-crop rotations preserved soil bulk density and larger aggregates better than continuous cropping. High-throughput amplicon sequencing of 16S rRNA gene and ITS fragments revealed that the pasture-crop rotations fostered taxa that are associated with soil structure maintenance and selected potential plant-beneficial bacterial genera in the oat rhizosphere (i.e. *Bosea*, *Devosia* and *Microbacterium*), that may have contributed to the observed increase in N uptake, N accumulation and biomass in oat. In summary, this study shows that pasture-crop rotations are an ecologically sustainable alternative to continuous cropping in the Uruguayan Pampa biome.

## 1. Introduction

The soil's capacity to function as living system, i.e. to sustain and promote plant and animal health as well as productivity and maintain environmental quality, has been described as soil health (Larkin, 2015; Banerjee and van der Heijden, 2023). The evaluation of soil health has been traditionally performed based on physical and chemical indicators or proxies for microbial parameters (Banerjee and van der Heijden, 2023). This "black box" approach neglected the role of soil microorganisms as engineers of soil structure and key players of many soil functions such as organic matter decomposition, nutrient cycling and soil suppressiveness (Kibblewhite et al., 2008; Banerjee and van der

Heijden, 2023). Moreover, the soil microbiome represents the pool from which the plant assembles its rhizosphere microbiome via release of root exudates contributing to plant growth and health (Berg et al., 2017). The soil microbiome sustains soil health and determines crop health and productivity (Hirt, 2020).

There is increasing evidence that land use and management practices affect the diversity, composition and functionality of soil microbiomes (Mendes et al., 2015; Bender et al., 2016; Babin et al., 2019; Moreno et al., 2019; Cerecetto et al., 2021; Tomazelli et al., 2023). Land use refers to an area-specific land cover, management, and activities. The concept alludes to natural, agricultural, and urban settings. Cropland for crop production and grassland for animal feed are common agricultural

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land use types (Yu et al., 2017). Intensive agriculture implies higher levels of inputs, e.g. capital, labour, agrochemicals, water, and outputs, e.g. higher biomass extraction, soil nutrient depletion. Therefore, intensive agriculture (e.g. continuous cropping, CC) leads in the long term to soil degradation and a loss of ecosystem functions (Kopittke et al., 2019). Low crop diversity can lead to an increased vulnerability to pests, weed invasion, and decreased yield production and soil health in the long term (Kopittke et al., 2019). Conservative agriculture can counter the negative effects of intensive agriculture and improve soil health. Minimal physical and chemical soil disturbance, permanent soil cover, no-till, crop rotation, and diversification are key components of conservative agriculture (Johansen et al., 2012; Schmidt et al., 2019b).

The Pampa biome, located in Uruguay, central-eastern Argentina and in the southern part of Brazil, is one of the most extensive natural grasslands in the world, with an area of 70 million hectares (Scottá and da Fonseca, 2015). The Pampa biome is dominated by grasses with sparse shrub and tree formations and hosts extensive biodiversity, with approximately 2200 plant species (Scottá and da Fonseca, 2015). Over the last two centuries, the main economic activity in this region has been livestock production, with natural grasslands as the main food source for cattle and sheep livestock (Roesch et al., 2009; Scottá and da Fonseca, 2015). In Uruguay, natural grasslands are traditionally combined with pasture-crop rotations to provide both grain and forage. Since the early 2000s, however, grain crop production has undergone intensification, characterized by the conversion of natural grasslands into cropping systems dominated by soybean (*Glycine max* L.). This intensification was stimulated by the increasing global demand for grains, and was facilitated by the massive adoption of no-till technology along with the use of glyphosate-resistant transgenic soybean cultivars (Rovira et al., 2020).

Conservative agricultural practices, such as mixed systems, which alternate in time grain crop cultivation and grazing livestock production in the same area, are regarded as a sustainable strategy to promote agricultural resilience while supporting high crop yields. This can be achieved via pasture-crop rotations, which increase landscape diversity, improve land use efficiency, livestock and agricultural productivity, and reduce soil erosion and degradation (Franco et al., 2021; Pereyra-Goday et al., 2022). Crop diversification, combining nitrogen-fixing legumes and cereals in the rotation, is another crucial factor influencing the C and N cycling in agricultural soils (McDaniel et al., 2014). Organic nutrient inputs either from livestock urine and faeces or from stubble provide environmental benefits to the pasture-crop system, such as increased soil fertility, C sequestration and improved physicochemical properties (Pravia et al., 2019; Bano et al., 2021; Pereyra-Goday et al., 2022). Grain-livestock systems are common in North and South America (Franzluebbers et al., 2014), Australia (Hochman et al., 2013) and Europe (Peyraud et al., 2014). In Uruguay, these systems represent 10% of the total livestock production area (excluding dairy cattle), occupying 2,159,000 ha (Rovira et al., 2020).

Although pasture-crop rotations have been shown to enhance soil health in terms of physicochemical indicators or proxies for microbial parameters (Ernst et al., 2018; Martin et al., 2020; Rubio et al., 2022; Santos Silva et al., 2022), knowledge about how they affect the relationship between microorganisms and crop performance is limited. Leveraging the effects of the conservative practices on soil and rhizosphere microbiome by enriching plant-beneficial microorganisms has been postulated as a promising new strategy for more sustainable agriculture (Bender et al., 2016; Bano et al., 2021), yet the mechanisms responsible for the effect of farming practices on the soil and rhizosphere microbiomes in the long term remained unclear.

Thus, this study aimed to assess how vegetation diversity and intensification in the Uruguayan Pampa biome affected the microbiota of the soil and oat rhizosphere, oat productivity, and, therefore, soil health. A 23-year-old long-term field experiment (LTE) used for beef cattle and grain production under no-tillage in Treinta y Tres, Uruguay, served as study site providing a gradient of land use intensities and vegetation diversity (Tables 1, S1; Fig. 1). The highest intensity and lowest vegetation diversity is CC consisting of two seasonal grain crop cultivations per year representing the highest amount of soil disturbance per rotation cycle, i.e., physical disturbance with machinery and chemical disturbance with agrochemicals, and with the lowest vegetation diversity (Tables 1, S1; Fig. 1). Short and long pasture-crop rotations with different pasture vegetation (SR and LR) were chosen as intermediate conservative practices (Tables 1, S1; Fig. 1). SR consists of two years of grain crop cultivation alternating with two years of less diverse sown pastures. LR consists of two years of grain crop cultivation alternating with four years of a more diverse pasture vegetation. LR has less physicochemical soil disturbance per rotation cycle and higher vegetation diversity than SR and CC. Natural grassland (NG) used for cattle grazing without crop cultivation was the reference used for conservative practice without any external input (Tables 1, S1; Fig. 1).
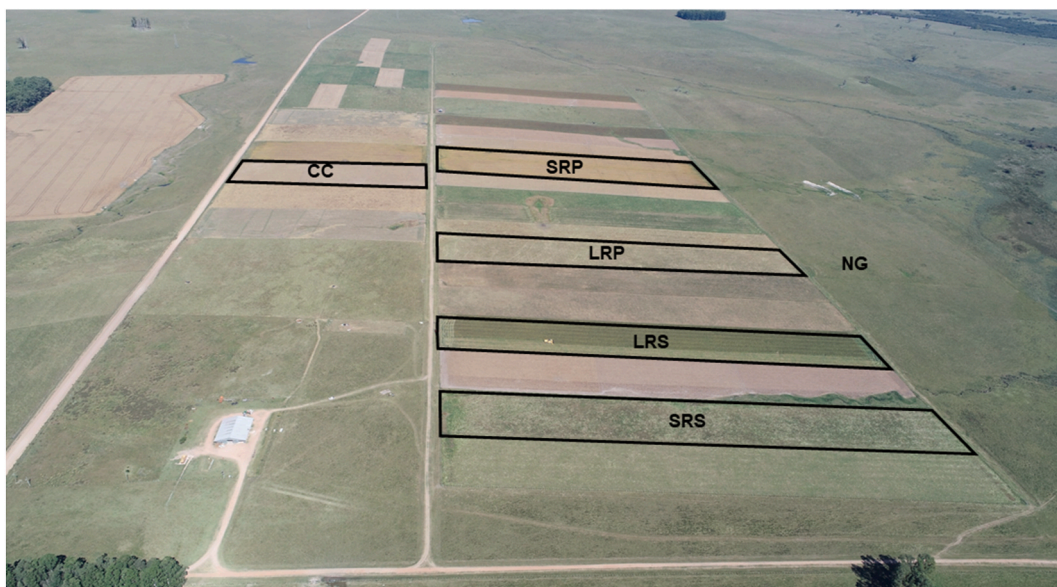


**Fig. 1.** Aerial view of the studied long-term field experiment. CC (Continuous Cropping), SRS (Short pasture-crop Rotation one year after pasture), LRS (Long pasture-crop Rotation one year after pasture), LRP (Long pasture-crop Rotation immediately after pasture), SRP (Short pasture-crop Rotation immediately after pasture), NG (Natural Grassland).

Additionally, two different stages of the rotation in SR and LR were assessed to study how long the pasture legacy lasts, comparing the next stage of the rotation immediately after the pasture (SRP and LRP) and one year later (SRS and LRS) (Tables 1, S1; Fig. 1). We hypothesized that (i) pasture-crop rotations preserve soil physicochemical parameters and select for plant-beneficial soil and rhizosphere microbiota supporting soil health and grain production in the Uruguayan Pampa biome; and (ii) the pasture legacy lasts through the whole grain crop cycle. To test these hypotheses, soil health in the different land use intensities and vegetation diversities was assessed based on physicochemical and biological properties including bulk density, aggregate size distribution, microbial (Bacteria/Archaea and Fungi) community diversity and composition and crop performance.

## 2. Material and methods

### 2.1. Experimental site

The long-term field experiment (LTE) is located at the National Institute of Agricultural Research (INIA) Palo a Pique Research Unit (33°15′54.4″ S 54°29′28.1″ W, elevation 60 m), Treinta y Tres, Uruguay. It was established in 1995 on a slightly degraded soil after a short history of soybean cropping with conventional tillage in the 1980s followed by a pasture of *Lolium multiflorum* L., bird's-foot trefoil (*Lotus corniculatus* L.), white clover (*Trifolium repens* L.) and the invasive species *Cynodon dactylon* (L.) Pers. (Pravia et al., 2019). The dominant soil type is Typic Argiudol with clay loam texture (22 % clay, 39 % silt, 39 % sand), 2 % soil organic carbon (SOC), a pH of 5.2 in the first 15 cm and a strong argillic Bt horizon conferring a poor internal water drainage. The erosion risk is high to moderate since the landscape has gently sloping hills of modest altitude. The region has a temperate sub-humid climate with an annual mean accumulated rainfall of 1379 mm without seasonality, and an annual mean maximum and minimum air temperature of 23 °C and 11.3 °C, respectively (Rovira et al., 2020).

The LTE evaluates different land use intensities and vegetation diversity under no-tillage (Fig. 1). The intensity ranges from CC (highest) to SR, LR, and NG (lowest), and the vegetation diversity in the agricultural plots ranges from LR (highest) to SR, and CC (lowest). In this study, intensity is defined by the years under grain crop cultivation, as this is the time in the rotation cycle with the highest physicochemical soil disturbances and inputs. In SR and LR the intensity decreases while a phase of sown pastures is integrated into the rotation (Table 1). The pasture vegetation composition is an integral part of the SR and LR strategy accounting for the different pasture duration times. In SR, the two-year pasture is composed of red clover (*Trifolium pretense* L.) and wheat (*Triticum aestivum* L.). In LR with four-year pasture, pasture plant species with a lasting persistence are used, including tall fescue (*Festuca arundinacea* L.), white clover and bird's-foot trefoil (Rovira et al., 2020) (Table S1). NG was selected as a reference for a soil with minimum disturbances, since only cattle grazing is allowed, while no tillage, fertilization or herbicides were applied in this area (Table 1).

Winter grain crops, including wheat and oat (*Avena sativa* L.), and summer grain crops, including sorghum (*Sorghum bicolor* L.) and soybean, are used for grain production in CC, LR and SR (Table S1). Pastures in SR, LR and NG are used for beef cattle production and mimic a commercial farm, with animals of the same age and body weight rotationally grazing. During the grain cropping phase, grazing is excluded from SR and LR plots. At the time of sampling, the NG consisted of 80–90 % *Cynodon dactylon* (L.) Pers. and 10–20 % of a mix of *Paspalum dilatatum* Poir, *Bothriochlo alaguroides* (DC) Herter, *Sporobolus indicus* (L.) R. Br., *Paspalum notatum* Flüggé and *Schizachyrium spicatum* (Spreng.) Herter.

Management practices, including machinery operations, fertilization, and agrochemical applications, were similar among CC, SR, and LR in the grain crop phase and mimic a commercial farm (Terra et al., 2006). Oat, which was the sampled grain crop in this study, was

**Table 1**

Description of the studied land use intensities and vegetation diversities in the LTE. CC (Continuous Cropping), SRS (Short pasture-crop Rotation one year after pasture), LRS (Long pasture-crop Rotation one year after pasture), LRP (Long pasture-crop Rotation immediately after pasture), SRP (Short pasture-crop Rotation immediately after pasture), NG (Natural Grassland), oat (*Avena sativa* L.), wheat (*Triticum aestivum* L.), red clover (*Trifolium pretense* L.), tall fescue (*Festuca arundinacea* L.), white clover (*Trifolium repens* L.), bird's-foot trefoil (*Lotus corniculatus* L.), NA (not applicable).

| Land use intensity and vegetation diversity | Years of pasture | Pasture vegetation | Stage of the rotation | Sampled crop |
|---|---|---|---|---|
| **CC** | 0 | NA | NA | oat |
| **SRS** | 2 | wheat, red clover | One year after pasture | oat |
| **LRS** | 4 | wheat, tall fescue, white clover, bird's-foot trefoil | One year after pasture | oat |
| **LRP** | 4 | wheat, tall fescue, white clover, bird's-foot trefoil | Immediately after pasture | oat |
| **SRP** | 2 | wheat, red clover | Immediately after pasture | oat |
| **NG** | Permanent | 80–90% *Cynodon dactylon* and 10–20% of a mix of *Paspalum dilatatum*, *Bothriochlo alaguroides*, *Sporobolus indicus*, *Paspalum notatum*, *Schizachyrium spicatum* | NA | NA |

fertilized with 10 kg ha$^{-1}$ of N, 30 kg ha$^{-1}$ of P$_2$O$_5$ and 30 kg ha$^{-1}$ of K$_2$O at sowing when grown immediately after pasture in SR and LR. Oat grown after sorghum in CC, SR and LR was fertilized with 8 kg ha$^{-1}$ of N, 13 kg ha$^{-1}$ of P$_2$O$_5$ and 8 kg ha$^{-1}$ of K$_2$O at sowing. Pastures in SR and LR were fertilized with 30 kg ha$^{-1}$ of N, 60 kg ha$^{-1}$ of P$_2$O$_5$ and 20 kg ha$^{-1}$ of K$_2$O at seeding and re-fertilized each fall with 50 kg ha$^{-1}$ of P$_2$O$_5$. Approximately 3.3 L ha$^{-1}$ of herbicide (glyphosate) was applied twice, two months before and immediately before sowing each grain crop in CC, SR and LR. NG has never received fertilizer or herbicide. Further details regarding the LTE and grazing management are described in Pereyra-Goday et al. (2022), Rovira et al. (2020) and Terra et al. (2006).

Sampling was carried out when oat (cultivar INIA-Columba; Tables 1, S1) was growing in CC, LR, and SR plots. Two different plots of SR and two different plots of LR were sampled at the same sampling time representing different stages of the rotation: SR-pasture and LR-pasture (SRP and LRP; oat grown immediately after pasture), and SR-sorghum and LR-sorghum (SRS and LRS; one year after pasture; Tables 1, S1). In total, five agricultural plots of 3 ha each were sampled: CC, SRS, SRP, LRS, and LRP, together with the NG located next to the LTE (Fig. 1).

### 2.2. Soil and plant sampling

Each agricultural 3 ha-plot was subdivided into six subplots which were randomly assigned along a slope gradient. From each subplot (replicate) soil and oat plants were sampled in October 2018 to determine soil physicochemical properties, bulk soil and rhizosphere microbiota, oat aerial biomass and nutrient content. Samples were collected when oat plants were mostly at their full flowering growth stage with plants ranging from beginning to end of flowering (BBCH stages 61–69) (Meier, 2018).

Bulk soil samples were taken from agricultural plots and NG. For chemical and microbial analyses, 15–20 soil core samples per replicate were randomly collected between plants with a 2 cm soil core auger at 0–15 cm depth, and mixed and homogenized by sieving with a 2 mm

mesh size to remove roots and stones. To determine soil aggregate size distribution, an undisturbed soil core ($20 \times 20 \times 20$ cm) was taken from each replicate. Two samples per replicate were taken from the 0–5 cm soil layer to determine bulk density using a soil core sampler (5 cm diameter $\times$ 5 cm depth).

Oat aerial biomass and rhizosphere samples were only taken from agricultural plots. All oat plants growing within one linear meter were collected at each replicate for estimating aerial biomass and nutrient content in plant tissue. Roots of three oat plants per replicate were sampled, briefly washed with distilled water to remove loosely adhering soil and pooled to a composite sample. The rhizosphere, defined as soil closely attached to roots, was retrieved from 5 g of roots by Stomacher treatment followed by centrifugation according to Schreiter et al. (2014).

Bulk soil and the pellets from oat rhizosphere samples used for microbial analysis were stored at $-20\ ^\circ$C until DNA extraction.

### 2.3. Aerial biomass and nutrient content in plant shoot

For aerial biomass dry weight, complete aboveground plant samples were oven-dried at 55 $^\circ$C and weighted afterwards. From each dried sample, a subsample was used to determine nutrient content. Plant carbon (C) was determined by 900 $^\circ$C combustion and subsequent $CO_2$ infrared detection technique. Plant nitrogen (N) was analyzed by combustion at 900 $^\circ$C and subsequent $N_2$ thermal conductivity detection. Plant phosphorus (P) was measured by sulfuric digestion and vanado-molybdate colorimetry. Plant potassium ($K^+$) was evaluated by dry digestion and atomic emission. Finally, calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), manganese ($Mn^{2+}$) and zinc ($Zn^{2+}$) were determined by dry digestion and atomic absorption.

### 2.4. Soil physicochemical properties

Bulk soil samples were first air-dried. Soil total N ($N_{tot}$) was analyzed by combustion at 900 $^\circ$C and subsequent $N_2$ thermal conductivity detection. Plant available P was measured by Bray-I method (Bray and Kurtz, 1945). Plant available $K^+$, $Ca^{2+}$ and $Mg^{2+}$ were evaluated by ammonium acetate (pH 7) extraction followed by atomic emission ($K^+$) or by atomic absorption ($Ca^{2+}$ and $Mg^{2+}$) (Jackson, 1964). SOC was quantified by 900 $^\circ$C combustion and subsequent $CO_2$ infrared detection (Wright and Bailey, 2001). Potentially oxidizable C (PoxC) was assessed by oxidation of a solution of 0.2 M $KMnO_4$ in 1 M $CaCl_2$ (pH 7.2) (Weil et al., 2003). The pH was measured by potentiometric determination in water (Beretta et al., 2014). Soil aggregate size distribution was determined according to Kemper and Chepil (1965). Bulk density (BD) was calculated after drying the soil at 105 $^\circ$C for 24 h (BD = soil core dry weight/soil core volume) (Lienhard et al., 2013).

### 2.5. DNA extraction and amplicon sequencing

DNA was extracted from 0.5 g of frozen bulk soil or frozen rhizosphere pellet (wet weight) by harsh lysis using a FastPrep-24 bead-beating system and the FastDNA Spin Kit for Soil (MP Biomedicals, USA) following the manufacturer's instructions. DNA quality was checked by agarose gel electrophoresis and stored at $-20\ ^\circ$C.

Soil and rhizosphere microbial communities were characterized by sequencing the V3–V4 region of the 16S rRNA gene for Bacteria/Archaea and the ITS2 region for Fungi. The V3–V4 region of the 16S rRNA gene was amplified according to Babin et al. (2019) with primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) (Sundberg et al., 2013). The ITS2 region was amplified according to Ihrmark et al. (2012) with primers gITS7 (5′-GTGARTCATCGARTCTTTG-3′) (Ihrmark et al., 2012) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Library construction and high-throughput amplicon sequencing was done by Novogene (Cambridge, UK) on an Illumina NovaSeq PE250 platform (Illumina, USA).

### 2.6. Processing of amplicon sequences

For 16S rRNA gene and ITS2 sequences, paired-end sequences were joined with an overlap of at least 10 bases using FLASH (Magoč and Salzberg, 2011). Sequences with a low quality score (Q < 20) were excluded, and the rest were denoised and chimeras were removed using DADA2 (Callahan et al., 2016) within QIIME2 (Caporaso et al., 2010) using *–p-trunc-len* 0. The reads were annotated with *classify-sklearn* (–p-confidence 0.7). Amplicon sequence variants (ASVs) (100 % sequence identity) were assigned using the SILVA v. 138 database for bacterial/archaeal 16S rRNA genes (Quast et al., 2013) and UNITE v 8.2 for fungal ITS (Nilsson et al., 2018; Kõljalg et al., 2020). Sequences affiliated to chloroplasts, mitochondria or unclassified at the domain level were discarded as well as ASVs with less than 10 reads.

Sequencing of 16S rRNA gene amplicons generated 5,919,482 high-quality sequences (between 55,576 and 113,187 reads per sample) and 18,002 ASVs. ITS2 sequencing generated 8,844,446 high-quality sequences (between 29,540 and 143,402 reads per sample) and 8960 ASVs. Rarefaction curves for both 16S rRNA gene and ITS2 sequences reached saturation (Fig. S1).

### 2.7. Statistical data analyses

Statistical analyses were done with R 4.2.2 software (https://www.r-project.org/) using packages *agricolae* (v1.3-5) (de Mendiburu, 2020), *phyloseq* (v1.42.0) (McMurdie and Holmes, 2013), *vegan* (v2.6–2) (Oksanen et al., 2022) and *ANCOM-BC* (v2.0.1) (Lin and Peddada, 2020; Lin et al., 2022). Graphics were prepared with the R packages *ggplot2* (v3.4.0) (Wickham, 2016), *pheatmap* (v1.0.12) (Kolde, 2019) and *corrplot* (v0.92) (Wei and Simko, 2021).

Data analyses (including rarefactions) were performed separately for each amplicon and soil compartment: bulk soil bacterial/archaeal samples were rarefied to 53,833; bulk soil fungal samples were rarefied to 58,565; rhizosphere bacterial/archaeal samples were rarefied to 68,293; and rhizosphere fungal samples were rarefied to 29,428. Alpha-diversity indices (richness, Pielou's evenness and Shannon's diversity) were calculated and averaged over a 999 times repeatedly subsampled data set.

A rank-based approach of land use intensity and vegetation diversity was used to perform a non-parametric multivariate analysis of variance (PERMANOVA) (Anderson, 2001) based on Bray-Curtis dissimilarities to test the effect of intensification and vegetation diversification on the microbial communities. NG was ranked as no intensity (0), LR as lowest intensity and higher diversity (1), SR as medium intensity and medium diversity (2), and CC as highest intensity and lowest diversity (3). Afterwards, pairwise PERMANOVAs were performed using a Benjamini-Hochberg correction. A principal coordinate analysis (PCoA) of Bray-Curtis dissimilarities was performed to visualize the communities. Heteroscedasticity of community assemblages using Bray–Curtis distance were tested by means of PERMDISP (Anderson, 2006). An analysis of the composition of microbiomes with bias correction (ANCOM-BC) (Lin and Peddada, 2020; Lin et al., 2022) was performed at the genus level to identify genera whose relative abundances consistently varied among land use intensities and vegetation diversities. Afterwards, logistic regression models were applied to each discriminant genus to assess its association with each land use intensity and vegetation diversity. Benjamini-Hochberg corrections were applied, and only models with corrected *p*-value < 0.05 were considered. Only the taxa significantly and positively associated with a specific land use intensity and vegetation diversity were considered in this study and termed unique responders.

Distance-based redundancy analyses (db-RDA) using Bray-Curtis dissimilarities were performed to assess the contribution of land use intensity and soil physicochemical properties at 0–15 cm depth to the

variation of microbial communities in bulk soils. Relevant parameters were selected using backward and forward selection (*ordiR2step* from the *vegan* package).

The relative abundances of the 15 most abundant microbial genera in bulk soil and rhizosphere were visualized in a heatmap in order to compare their abundance distributions. Spearman correlations were performed between the relative abundance of the 15 most abundant microbial rhizosphere genera, oat aerial biomass and shoot nutrient contents to explore the relationship between the microbial rhizosphere communities and plant properties. Only correlations with *p*-values < 0.05 and correlation coefficients > 0.5 were considered significant correlations. These significant correlations were explored with linear regression analyses.

The effects of intensification and vegetation diversification on oat biomass, nutrient content, soil physicochemical properties, and microbial alpha-diversity indices were statistically evaluated as described below. If normality or homogeneity of variance was violated, overall differences were assessed with Kruskall-Wallis tests, and pairwise differences with Wilcoxon rank sum-tests with Benjamini-Hochberg corrections. If assumptions were not violated, overall differences were evaluated with one-way ANOVAs, and pairwise differences with Tukey's HSD tests. Pearson correlations and linear regressions were performed between oat aerial biomass and shoot nutrient contents to explore the relationship between oat aerial biomass and plant nutrient contents. Significant correlations were defined as those having *p*-values < 0.05.

### 2.8. Accession numbers

Raw bacterial/archaeal and fungal sequences are available at NCBI Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) under the accession numbers PRJNA899016 and PRJNA945009, respectively.

## 3. Results

### 3.1. Pasture-crop rotation yielded higher oat biomass and better plant nutrient content

Intensification and vegetation diversification had a significant effect on oat aerial biomass and nutrient content (Fig. 2, Table S2). Oat aerial biomass was significantly correlated with shoot N and K content (Fig. S2). Oat aerial biomass and N content decreased gradually across the intensity gradient, from SRP and LRP (SR and LR immediately after pasture) to CC ($p_{\text{biomass}} < 0.001$, $p_{\text{N}} < 0.001$) (Fig. 2). The highest biomass and N content was observed when oat plants were grown in SRP, while SRS (SR one year after pasture) showed no differences from CC (Fig. 2). In contrast, $K^+$ content was higher in plants grown in CC, LRS and SRS in comparison to SRP and LRP ($p < 0.001$) (Table S1). The comparison with oat nutrient requirements from literature showed that P, $Ca^{2+}$ and $Mn^{2+}$ contents were in all land use intensities and vegetation diversities sufficient for oat growth; in contrast, only oat growing in SRP, LRP, and LRS met the N requirements for oat (Table S2).

### 3.2. Pasture-crop rotation preserved soil physical properties

Intensification and vegetation diversification affected soil bulk density and aggregate size distribution (all $p < 0.001$) (Fig. 3). Bulk density was lower in NG compared to all agricultural plots and increased from NG to CC ($p < 0.001$) (Fig. 3a). In comparison to CC, pasture-crop rotations maintained a lower bulk density (Fig. 3a), indicating that porosity was higher in these rotations. One year after pasture, bulk density increased compared to the stage immediately after pasture (SRS vs. SRP and LRS vs. LRP) (Fig. 3a).

The proportion of aggregates larger than 2 mm decreased gradually across the intensity and vegetation diversity gradient, from NG to CC ($p < 0.001$; Fig. 3b). These results are contrary to the proportion of small aggregates (< 0.25 mm, 0.25–0.5 mm, 0.5–1 mm) that increased from NG to CC ($p = 0.001$) (Fig. 3b). Within agricultural plots, SRP showed
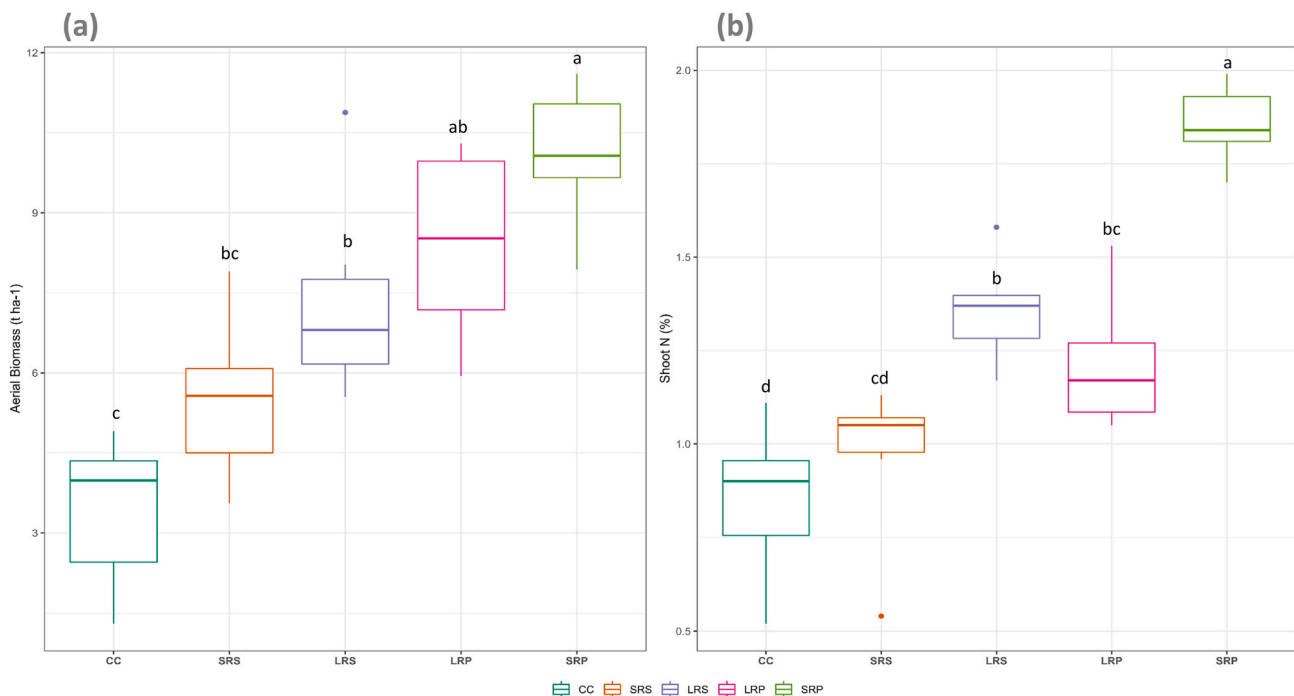


**Fig. 2.** Oat aerial biomass expressed as dry weight of shoot per hectare (a) and oat shoot N content (b). Data represent mean and standard deviation of six replicates. Significant differences among land use intensities and vegetation diversities are indicated by different letters, according to ANOVA-Tukey's HSD ($p < 0.05$). CC (Continuous Cropping), SRS (Short pasture-crop Rotation one year after pasture), LRS (Long pasture-crop Rotation one year after pasture), LRP (Long pasture-crop Rotation immediately after pasture), SRP (Short pasture-crop Rotation immediately after pasture).
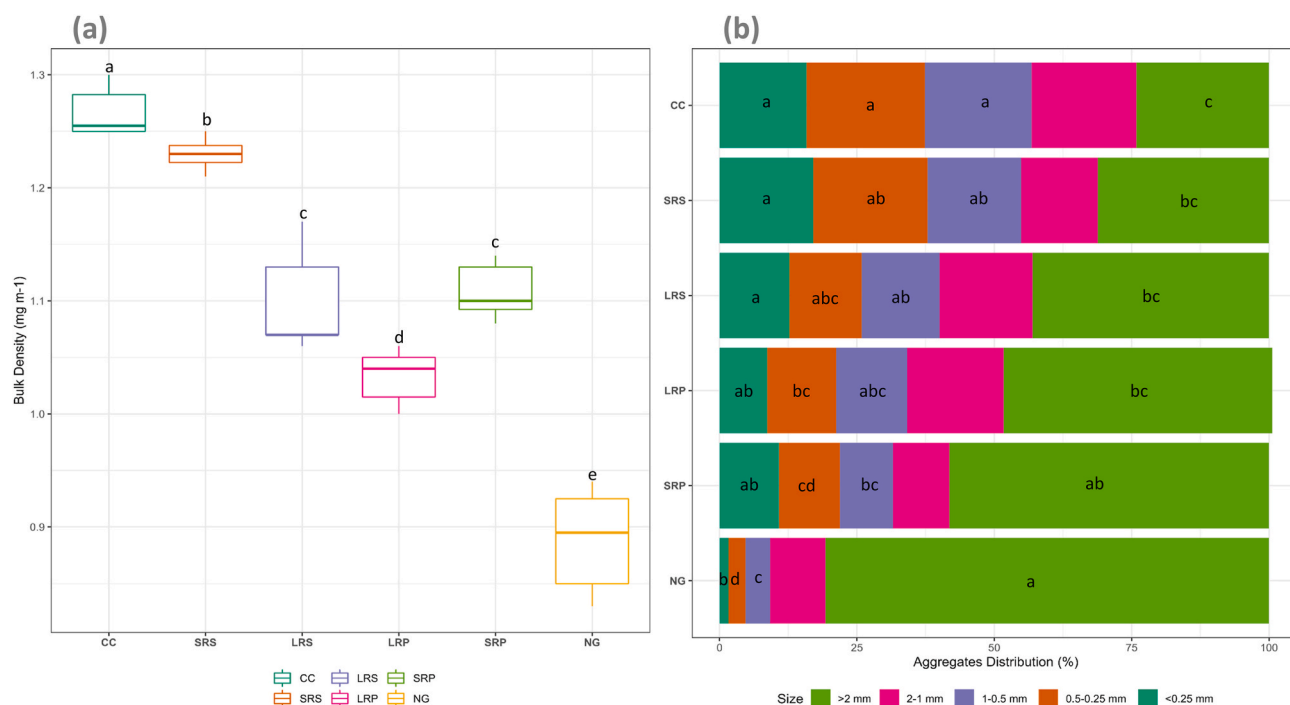
**Fig. 3.** Bulk density (a) and aggregate size distribution (%) (b) in bulk soil. Data represent mean and standard deviation of six replicates. Significant differences among land use intensities and vegetation diversities are indicated by different letters, according to ANOVA-Tukey's HSD ($p < 0.05$). Missing letters indicate no significant difference. CC (Continuous Cropping), SRS (Short pasture-crop Rotation one year after pasture), LRS (Long pasture-crop Rotation one year after pasture), LRP (Long pasture-crop Rotation immediately after pasture), SRP (Short pasture-crop Rotation immediately after pasture), NG (Natural Grassland).

the highest proportion of aggregates larger than 2 mm, and together with LRP the lowest proportion of small aggregates (Fig. 3b). Thus, aggregate size distribution in particular for SRP but also for LRP were less altered by agricultural practice compared to CC (Fig. 3b). However, one year after pasture, the aggregate size distribution is more similar to CC (SRS vs. SRP and LRS vs. LRP) (Fig. 3b).

### 3.3. High soil stratification and marginal differences in soil nutrients among land use intensities and vegetation diversity at 0–15 cm depth

For the majority of the measured soil nutrients, intensification and vegetation diversification effects were detected at 0–5 cm (Table S3), yet not at 0–15 cm (Table S4), indicating a high soil stratification across all plots due to the absence of tillage. At 0–5 cm, organic C was significantly higher in NG in comparison to all agricultural soils ($p < 0.001$). Even though there were no significant differences among the agricultural soils, organic C tended to be higher in the pasture-crop rotations compared to CC (Table S3). At a depth of 0–5 cm, PoxC was highest in LRS and lowest in SRP; however, at a depth of 0–15 cm, no significant differences between land use intensities and vegetation diversity strategies were observed (Tables S3 and S4). Soil N at 0–5 cm was higher in SRP in comparison to CC ($p = 0.04$) (Table S3). The pH was also significantly different among land use intensities and vegetation diversities at both depths. At 0–5 cm, the pH was higher in NG and CC compared to SRS, LRP and SRP ($p < 0.001$) (Table S3), and at 0–15 cm the pH was higher in NG in comparison to SRS, LRP and SRP ($p = 0.004$) (Table S4). At 0–15 cm, only available P, $K^+$ and $Mg^{2+}$ were significantly different among land use intensities and vegetation diversities (Table S4). Specifically, available P increased in agricultural soils in comparison to NG at both sampling depths ($p < 0.001$) (Tables S3 and S4). $K^+$ was higher in NG in comparison to SRP and LRP when looking at a depth of 0–15 cm ($p = 0.02$) (Table S4).

### 3.4. Intensification and vegetation diversification affected the diversity of bulk soil and rhizosphere microbial communities

Intensification and vegetation diversification affected the microbial alpha diversity in bulk soils, but not in the rhizosphere (Fig. S3). In bulk soil, bacterial/archaeal species richness, Pielou's evenness and Shannon's diversity increased gradually across the intensity and vegetation diversity gradient, from NG to CC (all $p < 0.01$) (Fig. S3). These results are contrary to fungal alpha diversity indices, where species richness and Shannon's diversity were higher in NG compared to pasture-crop rotations but did not significantly differ from CC (both $p < 0.01$) (Fig. S3).

The bulk soil microbial community composition structure was significantly affected by intensification and vegetation diversification (rank-based PERMANOVA) (Table S5). NG, SRP and LRP bacterial/archaeal communities differed from the other land use intensities and vegetation diversities ($p < 0.05$) (Table 2) and formed individual clusters in the PCoA (Fig. S4a). LRS and SRS communities did not differ (Table 2). Moreover, LRS did not differ from CC ($p = 1.00$). However, LRP and SRP communities were different from the CC community (Table 2). In the case of bulk soil fungal communities, NG significantly differed from LRS, SRP, and SRS ($p < 0.04$), while other land use intensities and vegetation diversities did not differ (Table 2, Fig. S4b). In the rhizosphere, the bacterial/archaeal, but not the fungal community composition structure was significantly affected by the intensification and vegetation diversification (rank-based PERMANOVA) (Table S5). Pairwise PERMANOVA tests revealed that bacterial/archaeal rhizosphere community differed in oat grown in LRP vs. SRP and LRP vs. CC ($p < 0.05$) (Table 2). All performed PERMDISP tests were non-significant, indicating that communities did not differ in dispersion and that the differences were entirely due to differences in intensification and vegetation diversification (Table S6).

**Table 2**
Land use intensity and vegetation diversification effect on bulk soil and oat rhizosphere microbial communities tested by pairwise non-parametric multivariate analysis of variance (PERMANOVA). * indicates significant differences ($p < 0.05$). CC (Continuous Cropping), SRS (Short pasture-crop Rotation one year after pasture), LRS (Long pasture-crop Rotation one year after pasture), LRP (Long pasture-crop Rotation immediately after pasture), SRP (Short pasture-crop Rotation immediately after pasture), NG (Natural Grassland).

| | Bacteria/Archaea | | | | Fungi | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Bulk Soil | | Rhizosphere | | Bulk Soil | | Rhizosphere | |
| | Explained variance (%) | adjusted *p*-value | Explained variance (%) | adjusted *p*-value | Explained variance (%) | adjusted *p*-value | Explained variance (%) | adjusted *p*-value |
| NG *vs.* CC | 31.6 | **0.030** * | – | – | 24.9 | 0.090 | – | – |
| SRP *vs.* CC | 23.4 | **0.015** * | 10.8 | 1.000 | 18.4 | 0.075 | 8.0 | 1.000 |
| LRP *vs.* CC | 25.7 | **0.015** * | 12.8 | **0.040** * | 18.3 | 0.075 | 6.8 | 1.000 |
| LRS *vs.* CC | 10.4 | 1.000 | 10.4 | 1.000 | 10.4 | 1.000 | 8.8 | 1.000 |
| SRS vs. CC | 12.0 | **0.045** * | 12.1 | 0.780 | 14.7 | 1.000 | 13.0 | 1.000 |
| SRP vs. SRS | 23.3 | **0.015** * | 13.6 | 0.400 | 15.9 | 0.810 | 8.7 | 1.000 |
| LRP vs. LRS | 19.4 | **0.045** * | 10.7 | 1.000 | 9.2 | 1.000 | 6.3 | 1.000 |
| SRP vs. LRP | 37.8 | **0.015** * | 14.3 | **0.030** * | 15.9 | 0.330 | 15.9 | 0.27 |
| SRS vs. LRS | 13.6 | 0.075 | 12.7 | 1.000 | 11.7 | 1.000 | 8.1 | 1.000 |
| SRP vs. LRS | 22.5 | **0.015** * | 23.5 | 1.000 | 21.1 | 0.105 | 4.2 | 1.000 |
| LRP vs. SRS | 26.5 | **0.045** * | 15.5 | 0.400 | 13.9 | 1.000 | 11.8 | 1.000 |
| SRP vs. NG | 33.0 | **0.015** * | – | – | 27.4 | **0.015** * | – | – |
| LRP vs. NG | 32.8 | **0.015** * | – | – | 22.5 | 0.090 | – | – |
| LRS vs. NG | 26.0 | **0.015** * | – | – | 27.3 | **0.015*** | – | – |
| SRS vs. NG | 30.1 | **0.015** * | – | – | 25.8 | **0.030** * | – | – |

### 3.5. More distinct bacterial/archaeal differentially abundant taxa among land use intensities and vegetation diversities in bulk soil than in rhizosphere

Intensification and vegetation diversification affected the composition of the bulk soil bacterial/archaeal community at phylum and genus level (Figs. S5a, 4a, S6a). In total, 407 bacterial/archaeal genera responded significantly to the different land use intensities and vegetation diversities in bulk soil (ANCOM-BC) (all $p < 0.05$). Fifty-eight land use intensity unique responders were identified in bulk soil using multiple logistic regression models (all $p < 0.05$) (Table S7). In Fig. 4a only unique responders with a relative abundance > 1 % are shown. Multiple unique responders to NG were detected, e.g. *Acinetobacter* (Proteobacteria), *Pseudomonas* (Proteobacteria), *Rhodococcus* (Actinobacteria), *Paenibacillus* (Firmicutes) (Fig. 4a). *Bacillus* (Firmicutes) was one of the genera identified as unique responders to SRP (Fig. 4a), together with other genera found in lower abundance, e.g. *Mucilaginibacter* (Bacteroidetes), *Ramlibacter* (Proteobacteria), *Microlunatus* (Actinobacteria), *Nitrospira* (Nitrospirae), *Granulicella* (Acidobacteria) (Table S7). Several unique responders to LRP were found, e.g. *Micromonosporaceae* (Actinobacteria), *Candidatus Udaeobacter* (Verrucomicrobia), *Pseudonocardia* (Actinobacteria), *Nitrososphaeraceae* members (Thaumarchaeota) (Fig. 4a). Three Actinobacteria, *Kitasatospora*, *Streptomycetaceae* and *Solirubrobacteraceae* members, were determined as unique responders to SRS (Fig. 4a). *Rhizobacter* (Proteobacteria), *Geobacter* (Proteobacteria), and *Bryobacter* (Acidobacteria) were identified as unique responders to CC (Fig. 4a). In bulk soil, no unique responders to LRS were found (Fig. 4a, Table S7).

In the rhizosphere, the intensification and vegetation diversification effects on bacterial/archaeal phyla and genera were less pronounced than in bulk soil (Figs. S5c, 4b, S6c). Two hundred ninety-five bacterial/archaeal genera responded significantly to the different land use intensities and vegetation diversities in the oat rhizosphere (ANCOM-BC) (all $p < 0.05$). However, only four unique responders were identified when using multiple logistic regression models (all $p < 0.05$) (Fig. 4b, Table S8). *Bosea* (Proteobacteria) and *Devosia* (Proteobacteria) were found as unique responders to SRP (Fig. 4b). *Janthinobacterium* (Proteobacteria) and *Solibacillus* (Firmicutes) were determined as unique responders to LRS and SRS, respectively (Fig. 4b). In the rhizosphere, there were no unique responders to LRP and CC (Fig. 4b, Table S8).

For a further description of the taxonomic composition of bacterial/archaeal bulk soil and oat rhizosphere communities, please see Supplementary Material (Text S1, S2; Figs. S5 and S6).

### 3.6. Intensification and vegetation diversification impacted fungal taxa to a lower extent than bacterial taxa

Intensification and vegetation diversification affected the bulk soil fungal community composition at genus level (Fig. 4c and S6b) and, to a lower extent, at phylum level (Fig. S5b). In total, 327 genera responded significantly to the different land use intensities and vegetation diversities in the bulk soil (ANCOM-BC) (all $p < 0.05$). Among them, 63 unique fungal responders were identified (all $p < 0.05$) (Table S9). However, only 24 exhibited a relative abundance of > 1 % (Fig. 4c). *Phialophora* (Ascomycota), *Neoascochyta* (Ascomycota), *Paraphaeosphaeria* (Ascomycota), among others, were found as unique responders to NG (Fig. 4c). Several unique responders to SRP were detected, e.g. *Fusarium* (Ascomycota), *Plectosphaerella* (Ascomycota), *Alternaria* (Ascomycota) (Fig. 4c). *Chaetomiaceae* and *Chaetothyriaceae* (both Ascomycota) members, among others, were observed as unique responders to LRP, and *Acremonium* (Ascomycota) as unique responder to LRS (Fig. 4c). Furthermore, several unique responders to CC were
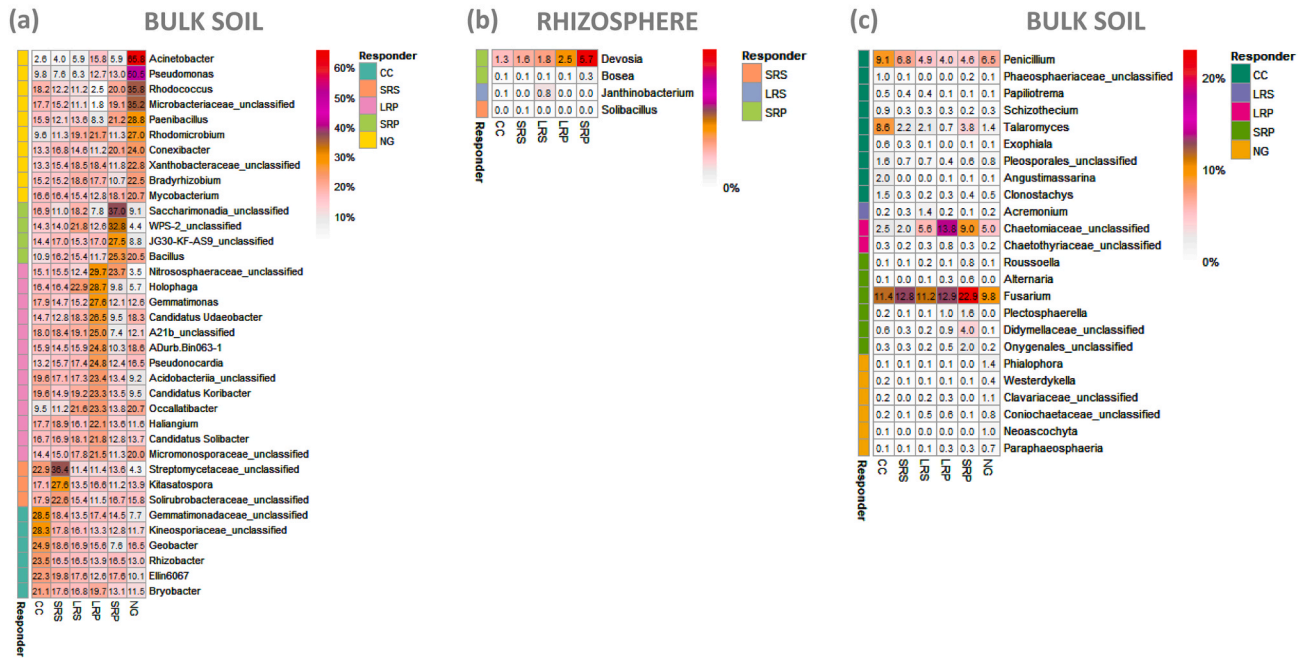
**Fig. 4.** Bacterial/archaeal unique responders to each land use intensity and vegetation diversity in bulk soil (a) and oat rhizosphere (b), and fungal unique responders in bulk soil (c), determined by analysis of composition of microbiomes with bias correction (ANCOM-BC) at the genus level and logistic regression models applied to each discriminant genus. Colours and numbers indicate mean relative abundance (n = 6) of each taxon. In (a) and (c) only taxa with relative abundances > 1 % are shown. No fungal unique responders in oat rhizosphere were found. CC (Continuous Cropping), SRS (Short pasture-crop Rotation one year after pasture), LRS (Long pasture-crop Rotation one year after pasture), LRP (Long pasture-crop Rotation immediately after pasture), SRP (Short pasture-crop Rotation immediately after pasture), NG (Natural Grassland).
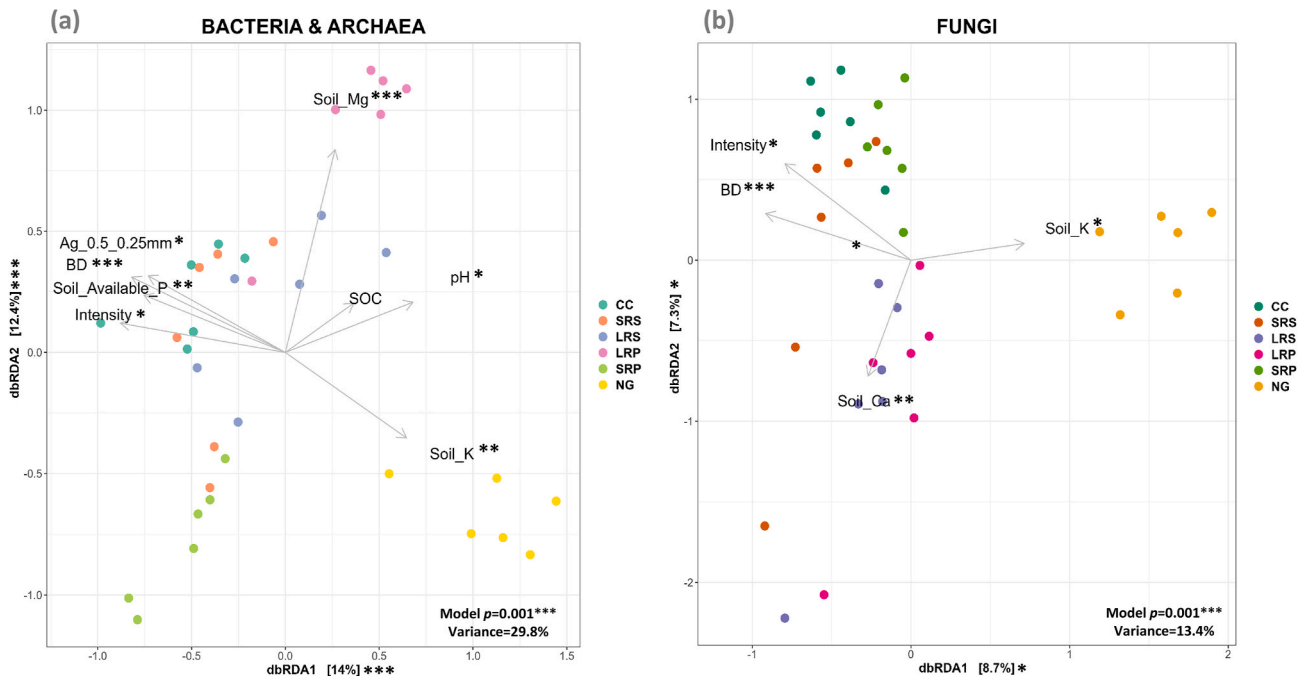


**Fig. 5.** Distance-based redundancy analyses (db-RDA) based on Bray-Curtis dissimilarity indices of bacterial/archaeal (a) and fungal (b) bulk soil communities. The soil physicochemical properties data set used to perform this analysis was obtained from 0 to 15 cm soil depth. Significance of model, axes and factors were determined by ANOVA. CC (Continuous Cropping), SRS (Short pasture-crop Rotation one year after pasture), LRS (Long pasture-crop Rotation one year after pasture), LRP (Long pasture-crop Rotation immediately after pasture), SRP (Short pasture-crop Rotation immediately after pasture), NG (Natural Grassland), BD (Bulk Density), SOC (Soil Organic Carbon), Ag_0.5_0.25 mm (percentage of aggregates of 0.5–0.25 mm), * ($p < 0.05$), ** ($p < 0.01$), *** ($p \leq 0.001$).

determined, e.g. *Penicillium* (Ascomycota), *Angustimassarina* (Ascomycota), *Clonostachys* (Ascomycota), *Papiliotrema* (Basidiomycota) (Fig. 4c).

In total, 108 fungal genera responded significantly to the different land use intensities and vegetation diversities in the rhizosphere (ANCOM-BC) (all $p < 0.05$), however, no unique fungal responders were found after applying multiple logistic regression models.

For a further description of the taxonomic composition of fungal bulk soil and oat rhizosphere communities, please see Supplementary Material (Text S1, S2; Figs. S5 and S6).

### 3.7. Linking microbiota and soil physicochemical properties

In order to understand the contribution of land use intensity and soil physicochemical properties to the differentiation in bulk soil microbial community composition among land use intensities and vegetation diversities, db-RDA analyses were carried out. In the case of bulk soil bacterial/archaeal communities, intensity, bulk density (BD), aggregates of 0.5–0.25 mm size, soil available P, soil $Mg^{2+}$, soil $K^+$, pH and SOC at 0–15 cm depth were selected to perform the db-RDA after using backward and forward selection, and explained 29.8 % of total variance (Fig. 5a). NG and LRP formed two individual clusters due to positive correlation with either soil $K^+$ or soil $Mg^{2+}$, respectively (Fig. 5a). Intensity, bulk density, soil available P and aggregates of 0.25–0.5 mm size were positively correlated with bacterial/archaeal communities in CC and negatively with NG, contributing to their separation along axis 1 (Fig. 5a). In the case of bulk soil fungal communities, the db-RDA explained only 13.4 % of total variance which was attributed to intensity, bulk density, $Ca^{2+}$ and $K^+$ contents in soil at 0–15 cm depth (Fig. 5b). The first axis separated the agricultural soils from NG (Fig. 5b). Soil $K^+$ positively correlated with fungal communities in NG. Intensity

and bulk density contributed to the separation of SRS, SRP and CC, while soil $Ca^{2+}$ was positively correlated with fungal communities in LRS and LRP (Fig. 5b). For both bacteria/archaea and fungi, stochastic factors or variables that were not determined had a high impact since 70.2 and 86.6 % of total variance, respectively, was not explained by parameters measured.

### 3.8. Devosia, Microbacterium and Pyrenochaetopsis positively correlated to oat aerial biomass

The relationship between plant aerial biomass and shoot nutrients and the relative abundance of the 15 most abundant microbial genera in the oat rhizosphere was explored by Spearman's correlations (Fig. 6) and the most crucial correlations through linear regression analyses (Fig. S7). Only correlations with $p$-values $< 0.05$ and correlation coefficients $> 0.5$ were considered significant correlations. *Devosia* was significantly positively correlated with plant aerial biomass and N, and significantly negatively correlated with plant $K^+$ (Fig. 6a). *Microbacterium* was also significantly positively correlated with plant aerial biomass (Fig. 6a). Interestingly, these two genera were also significantly increased in SRP in comparison to CC (Fig. 6a). The fungal genus *Pyrenochaetopsis* was significantly positively correlated with plant aerial biomass (Fig. 6b). This genus was significantly increased in SRP and LRP in comparison to CC, SRS and LRS (Fig. 6b). *Cystofilobasidium* was significantly negatively correlated with plant aerial biomass (Fig. 6b).

### 4. Discussion

Healthy soils are an important resource for agricultural production (Banerjee and van der Heijden, 2023). Even though it has been demonstrated that pasture-crop rotations improve soil health (Ernst
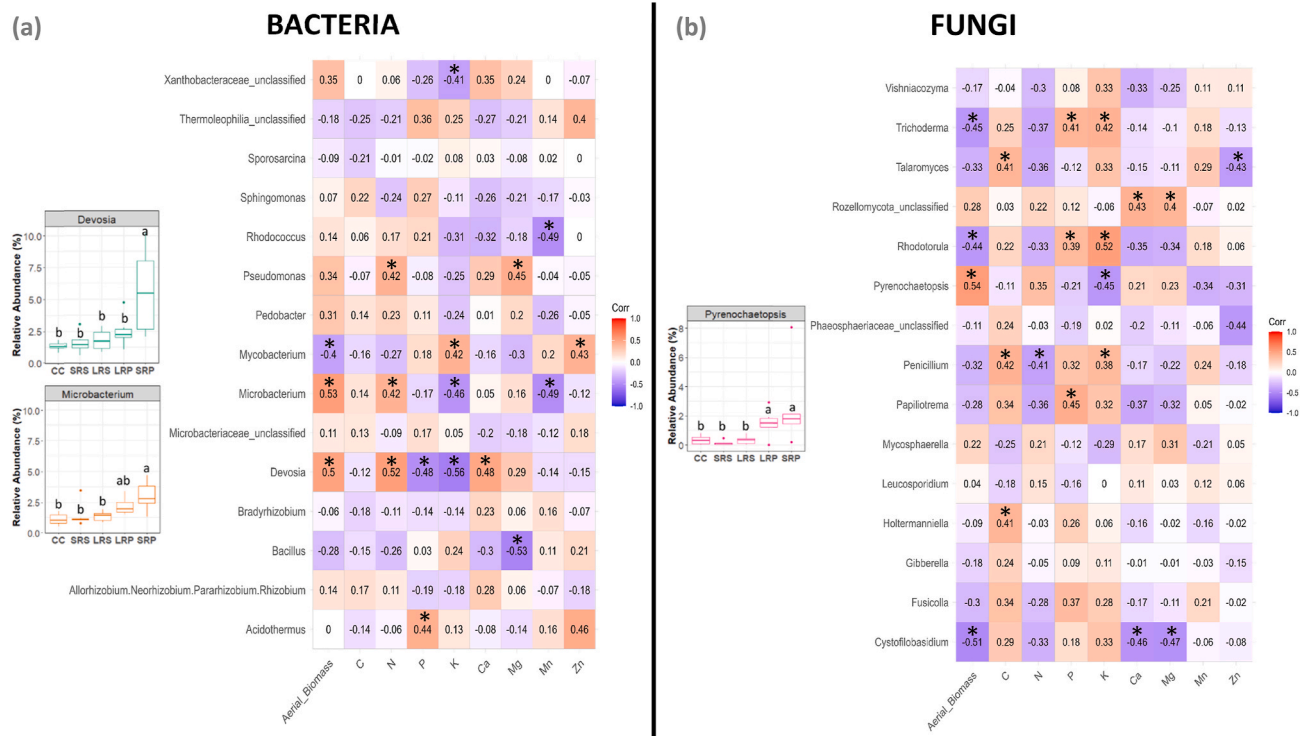


**Fig. 6.** Spearman correlation between the relative abundance of the top 15 abundant bacterial (a) or fungal (b) genera in the rhizosphere of oat and the aerial biomass and shoot nutrient contents of oat. * indicates significant correlations ($p < 0.05$). Relative abundances of selected genera in the different land use intensities and vegetation diversities are shown. Data represent mean and standard deviation of six replicates. Significant differences among land use intensities and vegetation diversities are indicated by different letters, according to ANOVA-Tukey's HSD ($p < 0.05$). CC (Continuous Cropping), SRS (Short pasture-crop Rotation one year after pasture), LRS (Long pasture-crop Rotation one year after pasture), LRP (Long pasture-crop Rotation immediately after pasture), SRP (Short pasture-crop Rotation immediately after pasture).

et al., 2018; Martin et al., 2020; Rubio et al., 2022; Santos Silva et al., 2022) and crop productivity (Nafziger and Dunker, 2011; Fontaneli et al., 2012; Franzluebbers et al., 2014; Pereyra-Goday et al., 2022) in comparison to intensive agriculture (i.e. CC), it remains unclear how these improvements relate to changes in the microbial community especially in terms of taxa beneficial for plant growth and health. To tackle this gap, this study explored the link between microbial community composition, soil health and oat performance across an intensification and vegetation diversity gradient in a LTE in the Uruguayan Pampa. The results indicate that the integration of a pasture phase preserved soil structure similar to NG, which served as a reference for a healthy soil. Soil structure preservation might in part be due to the observed pasture modulation of the soil microbial community. Furthermore, pasture-crop rotation promoted the relative abundance of potential plant-beneficial bacterial genera in the oat rhizosphere, i.e. *Bosea*, *Devosia* and *Microbacterium,* which positively correlated to oat aerial biomass and plant N content. Thus, these bacterial taxa might have enhanced N uptake and promoted N accumulation in oat, resulting in a biomass improvement and thereby supporting the study's first hypothesis. Conversely, the study's second hypothesis cannot be completely accepted. Shortly after the pasture phase, SR had the most favourable pasture effect on soil health and plant performance; however, this benefit gradually decreased over time. The length and diversity of the pasture phase likely influenced the pasture legacy. The legacy clearly persisted longer in the four-year pasture of *Gramineae* and legumes (LR) than in the two-year pasture of legumes mainly (SR).

### 4.1. Land use intensity and vegetation diversity affected microbial diversity and community composition

While microbial alpha-diversity is often linked with improved soil health (Bender et al., 2016), in this study, we found that soil bacterial/archaeal alpha-diversity was higher in CC than in NG and pasture-crop rotation soils. This is in line with Mendes et al. (2015), who reported a higher microbial diversity and functional redundancy in agricultural and pasture sites compared to forest in the Brazilian Amazon. Also Tomazelli et al. (2023) found that the change from NG in the Brazilian Pampa to cultivated pastures increased the bacterial alpha-diversity. Hence, although alpha-diversity was previously proposed as a crucial soil health indicator, microbial community composition matters more as shown in other recent studies (reviewed by Bender et al., 2016). Soil microbial communities with such high diversity hold high levels of functional redundancy. Thus, changes in the microbial community composition would not translate immediately into changes in functioning (Bender et al., 2016). Furthermore, our results are in line with the Intermediate Disturbance Hypothesis since diversity increased after CC (representing a moderate disturbance event), while NG (similar to an equilibrium state) harboured a lower diversity (Mendes et al., 2015).

The intensification and vegetation diversification effect on the bacterial/archaeal community composition was more pronounced than for fungi. Unlike the fungal community, the rhizosphere effect on the bacterial/archaeal did not override the pasture legacy effect on the community structure. Moreover, the pasture legacy effect on the bulk soil bacterial/archaeal community composition was time-dependent. Pairwise PERMANOVA tests showed that bacterial/archaeal communities in LRP and SRP were significantly different from LRS and SRS, respectively. Furthermore, LRS and SRS bacterial/archaeal communities were similar to CC, contrary to LRP and SRP, which differed from CC.

To our knowledge, this is the first study that addresses the effect of incorporating pasture into a crop rotation sequence on the soil and rhizosphere microbiota. Previous studies in the Pampa biome focused on either grassland or cropland. Tomazelli et al. (2023) studied how the soil bacterial community was affected by the conversion of NG to cultivated pastures. They found that the management intensification and pasture diversity shifted the bacterial diversity and composition.

Fernandez-Gnecco et al. (2021) showed that the cropping regime affected soil microbial communities. Figuerola et al. (2015) reported that beta-diversity decreased in monoculture compared to crop rotation and that good agricultural practices had a similar degree of beta-diversity compared to NG.

### 4.2. Effects of microbial community shifts on soil health and oat productivity

Oat aerial biomass at flowering stage was measured as plant growth indicator and was found to be increased in pasture-crop rotations in comparison to CC. This is in line with several studies of pasture-crop rotations in the US (Posner et al., 2008; Grover et al., 2009; Nafziger and Dunker, 2011), Brazil (Fontaneli et al., 2012) and Uruguay (Franzluebbers et al., 2014; Pereyra-Goday et al., 2022). Higher oat aerial biomass under pasture-crop rotations might be due to a combination of: (i) the improvement of soil structure and (ii) the increment in soil N fertility.

Pasture-crop rotations showed a positive effect on soil physical attributes, even in the absence of tillage. Pasture-crop rotations contributed to the preservation of bulk density and larger aggregates, in particular at the rotation stage immediately after pasture. Soil compaction is a major problem in agricultural soils (Jarvis et al., 2017; Shah et al., 2017; Longepierre et al., 2021). In this study, bulk density, which can be used as indicator for soil compaction, was highest in CC and decreased gradually to NG, highlighting the relationship between soil compaction and agricultural intensification and the role of pastures in preserving soil porosity. The perennial roots of grasses likely contributed to maintain a stable soil structure and greater porosity (Franzluebbers et al., 2014). Similarly, pasture-crop rotations resulted in higher SOC levels. SOC content in soil plays a key role for soil structure stabilization (Six et al., 2000a, 2000b). The permanent vegetation cover during the pasture phase likely increased the plant litter input, contributing to the observed improved preservation of larger soil aggregates. These results align with the general recognition of positive soil structure effects (Ball et al., 2005) and highlight the role of pasture rotations in safeguarding soil health.

Agricultural practices modify soil structure (e.g. aggregation and pore connectivity) and therefore the soil microbiota diversity and composition, by regulating the availability of water, oxygen and nutrients (Hartmann and Six, 2023). Particularly, bulk density explained most of the variance in bulk soil microbial communities among land use intensities and vegetation diversities. Thus, soil structure and microbiota are intimately linked and this influence can be bidirectional. Soil fungi play an important role in maintaining soil structure (Ritz and Young, 2004; O'Callaghan et al., 2022). Almost all of the unique soil fungal responders identified here are likely saprotrophs with filamentous growth. Therefore, from ITS responder analysis no clear indication for the fungal contribution to the observed differences in soil structure between land use intensities and vegetation diversities can be derived. The absence of differences in fungal functional traits among land use intensities and vegetation diversities may be due to the fact that this study was conducted in a no-till LTE, strongly reducing the physical disturbance, and with a crop rotation, providing organic C sources for decomposers (Schmidt et al., 2019a). Soil bacteria, in contrast, might have played a bigger role due to their ability to produce extracellular polymeric substances (EPS). This has been shown for strains belonging to e.g. *Bacillus* (Firmicutes) (Marvasi et al., 2010), *Mucilaginibacter* (Bacteroidetes) (Kumar et al., 2022), *Ramlibacter* (Proteobacteria) (Jivkova et al., 2022), *Microlunatus* (Actinobacteria) (Lima-Miranda et al., 2018), *Nitrospira* (Nitrospirae) (Spieck et al., 2006), and *Granulicella* (Acidobacteria) (Costa et al., 2020), which were found here in the bulk soil as unique responders to SRP (SR immediately after pasture). EPS can improve soil aggregation and the macroporosity of the soil (Zethof et al., 2020; Bettermann et al., 2021; O'Callaghan et al., 2022). In addition, two taxa of filamentous bacteria, i.e. *Micromonosporaceae*

(Actinobacteria) and *Pseudonocardia* (Actinobacteria), were found as responders to LRP, which could have contributed to the soil structure preservation. Similar, yet to lower extent than fungal hyphae, filamentous Actinobacteria are also involved in the formation of stable soil aggregates (Wolf et al., 2013; Shivlata and Satyanarayana, 2017). Furthermore, members of Actinobacteria and *Bacillus* are able to degrade complex organic compounds (Feto and Motloi, 2016; Moreno-Espíndola et al., 2018), increasing organic binding agents which might have contributed to the observed soil structure preservation in SRP and LRP (Ritz and Young, 2004).

However, the observed positive effects of pastures on bulk density and aggregate size distribution diminished with increasing temporal distance to the pasture phase (LRP/SRP vs. LRS/SRS). This is partly in line with Ernst et al. (2018), who found that water infiltration gradually reduced with increasing temporal distance to the pasture phase in a LTE in the northwestern part of the Uruguayan Pampa indicating a gradual degradation of soil physical properties. Yet, the legacy effect of the four-year pasture (LR) was lasting longer than the two-year pasture (SR) phase. This can be attributed on the one hand to the pasture duration and on the other hand to the different pasture vegetation composition. Furthermore, effects from machinery traffic on soil structure cannot be excluded (Alvarez et al., 2014), as LR has less traffic than SR due to the duration of the pasture where no machinery is used (four years in LR vs. two years in SR). In addition, the relative abundance of the putative EPS-producing taxa found as unique responders to SRP decreased in SRS, which may have added to the decline in soil structure.

Pastures also affected soil chemical parameters. Pasture-crop rotations and CC had more available P than NG, likely due to P fertilization. Additionally, the lower amount of $K^+$ in SRP and LRP compared to NG may be due to the fact that historically $K^+$ was not used as fertilizer, which is reflected in the low oat $K^+$ content. The reduction in soil pH in pasture-crop rotations in comparison to CC and NG may be due to N fertilization and N fixation, and is consistent with regional observations (Beretta-Blanco et al., 2019; Rubio et al., 2022). Pasture-crop rotations increased soil total N in comparison to CC, while SRP exhibited the highest amount. Ramirez et al. (2012) showed that increased activity of N-fixing and ammonia-oxidizing microorganisms under intercropping systems resulted in higher amounts of different N forms (e.g. total N, $NO_3^-$, $NH_4^+$) in soil. In the present study, some taxa comprising strains involved in N cycling were found as unique responders to pasture-crop rotations in bulk soil, e.g. *Bacillus* (Beneduzi et al., 2008; Gouda et al., 2018) in SRP, *Nitrososphaeraceae* (Stieglmeier et al., 2014) in LRP, *Streptomycetaceae* (Dahal et al., 2017) in SRS, which may have increased the soil total N content in pasture-crop rotations.

Consequently, oat growing in SRP and LR met the N requirements (Westfall et al., 1990) contrary to CC. Particularly, oat growing in SRP showed the highest aerial biomass and shoot N content. In SRP, two genera *Bosea* and *Devosia*, related to N metabolism, were identified as unique responders in the oat rhizosphere. *Bosea* and *Devosia* isolates had been reported to fix N, to produce enzymes related to N cycle, e.g. urease, nitrate and nitrite reductase, and to exhibit other plant-beneficial traits, e.g. indole acetic acid (IAA) and siderophore production (Rivas et al., 2002; Vanparys et al., 2005; Rincón et al., 2008; De Meyer and Willems, 2012; Rilling et al., 2018; Sazanova et al., 2019; Chhetri et al., 2022; Pulido-Suárez et al., 2022). Nevertheless, compared to *Devosia* (5.68 ± 3.48 %), *Bosea* was found in low relative abundance in the SRP oat rhizosphere (0.29 ± 0.20%). *Devosia* and *Microbacterium* correlated significantly positive with N and aerial biomass. *Devosia* displayed the highest relative abundance in SRP and *Microbacterium* in SRP and LRP. However, their abundance diminished in the next rotation phase and was similar to CC. *Microbacterium* isolates were reported to promote plant growth in different crops, through different mechanisms, e.g. N fixation, phosphate solubilization, IAA production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and siderophore production (Sheng et al., 2009; Madhaiyan et al., 2010; Lin et al., 2012). In addition, the lower proportion of legumes sown in the pasture phase of LR in

comparison to SR likely resulted in lower microbial N-fixation.

As for soil structure, the positive pasture legacy effect on oat biomass diminished with increasing temporal distance to the pasture phase. The shoot N content and oat biomass in SRP were higher than in SRS. However, this was not observed in LR. SR pasture is mainly composed of the legume red clover, while LR pasture is a mix of legumes and *Gramineae*. N fixation by symbiosis between legumes and bacteria provides N to non-legume crops of the rotation and/or to *Gramineae* of the pasture (Pravia et al., 2019). Given that greater amounts of N immobilization are expected for *Gramineae* litter compared to legume litter (Guinet et al., 2020), N mineralization takes longer than for legume litter. Therefore, N mineralization and availability in LR were likely more sustained over time than in SR contributing to the more stable oat aerial biomass and N shoot content.

## 5. Conclusions

This research demonstrated that the legacy of pasture positively affected the subsequent grain crop cycle through physical, chemical and microbial changes in the soil. The positive pasture effect on soil health and plant performance parameters was highest in the short rotation immediately after the pasture phase (SRP), yet diminished with time. The duration of the pasture phase and the diversity of pasture vegetation determined the extent of the pasture legacy. The legacy lasted longer in the four-year pasture composed of *Gramineae* and legumes (LR) than in the two-year pasture consisting mainly of legumes (SR). In order to benefit from pasture effects, farmers should take this into consideration. For instance, crops with high nutritional demand should be grown immediately after a pasture rich in legumes.

This study revealed that the positive pasture effect is likely also due to a modulation of the soil microbial community, since certain microbial taxa were fostered that may have contributed to the preservation of soil structure. Additionally, the enrichment of microbial taxa putatively involved in the N cycle (e.g. *Devosia*, *Microbacterium*, *Bosea*, *Nitrososphaeraceae*) might have increased soil N availability, enhanced N uptake and promoted N accumulation in oat, resulting in oat biomass improvement. In general, bacteria/archaea were more responsive to pasture-crop rotations than fungi.

In conclusion, this study highlights that pasture-crop rotation is an ecologically sustainable alternative to continuous cropping in the Pampa preserving soil health in particular with respect to soil structure, N fertility and supporting grain crop performance.

## CRediT authorship contribution statement

**Victoria Cerecetto:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – review & editing. **Carolina Leoni:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Stephanie D. Jurburg:** Writing – review & editing, Methodology, Formal analysis. **Ioannis D. Kampouris:** Writing – review & editing, Methodology, Formal analysis. **Kornelia Smalla:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Doreen Babin:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Data curation.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2024.109451.

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