Microbial community structure in rice, crops, and pastures rotation systems with different intensification levels in the temperate region of Uruguay

Sebastián Martínez

Instituto Nacional de Investigación Agropecuaria (INIA), Programa Nacional de Investigación en Producción de Arroz, Laboratorio de Patología Vegetal, Estación Experimental INIA Treinta y Tres, Ruta 8 km 281, Treinta y Tres, C.P. 33000, Uruguay.

Email: smartinez@inia.org.uy

Telephone (+598) 4452 2023 1216

Fax (+598) 4452 5701

Orcid 0000-0003-0455-5823

28 **Abstract**

29 Rice is an important crop in Uruguay associated mostly with livestock production in a
 30 rice and pasture rotation system since the 1920s. However, in recent years there has
 31 been interest in intensifying the production in some of these systems to satisfy market
 32 demands and increase income. Intensification occurs by augmenting the rice frequency
 33 in the rotation, including new crops like sorghum and soybean, or shortening the
 34 pasture phase. A long-term experiment was established in 2012 in the main rice
 35 producing area of Uruguay with the objective to study the impact of intensification in
 36 rice rotations. After the first cycle of rotation soils from seven rotation phases were
 37 sampled and microbial communities were studied by means of high-throughput
 38 sequencing of Illumina NovaSeq 6000. Archaeal/bacterial and fungal community
 39 composition were studied (16S rRNA and 18S gene regions) detecting 3662 and 807
 40 bacterial and fungal Operational Taxonomic Units (OTUs), respectively. *Actinobacteria*,
 41 *Firmicutes* and *Proteobacteria* were the most common bacterial phyla. Among them,
 42 only *Proteobacteria* differed significantly between rotations. Although most fungal
 43 OTUs were unidentified, *Ascomycota*, *Basidiomycota* and *Mucoromycota* were the
 44 most abundant fungal classes within identified taxa. Bacterial communities differed
 45 between rotations forming three groups according to the percentage of rice in the
 46 system. Fungal communities clustered in four groups, although not well differentiated,
 47 and mostly associated with the antecessor crop. Only P and C:N varied between
 48 rotations among soil physicochemical variables after six years, and individual bacterial
 49 OTUs appeared weakly influenced by P, pH, Mg and fungal OTUs by P. The results
 50 suggest that after six years, bacteria/archaeal communities were influenced by the time
 51 with rice in the rotation, and fungal communities were more influenced by the
 52 antecessor crop. More studies are needed to associate fungal communities with certain
 53 rotational or environmental variables. Some taxa were associated with a particular
 54 rotation, and some bacterial taxa were identified as biomarkers. Fungal indicator taxa
 55 were not identified at the species level for any rotation.

Keywords: Bacteria, Cover crops, Fungi, Long-term experiment, NovaSeq 6000,
Sustainable agriculture.

58

59 **1. Introduction**

60 Soil microorganisms mediate different and important ecosystem functions
61 associated with nutrient cycling, primary productivity, contaminant remediation, C cycle
62 processes and climate regulation (Bardgett and van der Putten, 2014). Soil microbial
63 diversity and processes are critical for maintaining sustainability and production of
64 diverse agricultural systems (Bending et al. 2004; Bünemann et al. 2018). In addition,
65 soils are reported to substantially impact microbial community diversity and structure
66 (Bending et al. 2004; McGuire and Treseder 2010). Several soil physicochemical and
67 biological factors are reported to influence the structure and diversity of soil
68 microbiomes, including pH, water availability, soil type, soil texture, weather conditions,
69 interactions with other underground and aboveground soil organisms, as well
70 anthropogenic, geological and historical factors (Bending et al. 2004; Brockett et al.
71 2012; Zheng et al. 2019). Agriculture is reported to cause disturbances in soils,
72 particularly affecting some of the physicochemical factors mentioned previously and,
73 therefore, the associated microbiota (Bending et al. 2004).

74 Rice (*Oryza sativa* L.) is one of the most cultivated crops feeding more than
75 50% of the world population and influencing the livelihoods and economies of several
76 billion people (IRRI, 2006). Soils subjected to irrigated rice cultivation differ from most
77 other crop soils in that they are subjected to oxic and anoxic phases within the crop
78 cycle. These changes result in the selection of specific physiological groups of
79 microorganisms correlated with chemical differences owing to their metabolism, being,
80 aerobic, anaerobic, or facultative (Brune et al., 2000). The physicochemical properties
81 show great variation among soil types found in different geographical locations, and,
82 thus, soil microbial communities change accordingly. Most reports focus on the

83 changes among microbial communities in soils with different nutritional statuses (Jiang
84 et al., 2016).

85 Several studies on microbial communities in paddy soils focused on the effects
86 of crop rotation, fertilization and residue retention on the soil microbial abundance,
87 biomass, composition and activity (Kim and Lee, 2020). But, most of these studies
88 focused on rice production systems in Asia where continuous rice, with two or three
89 crops each year, is substituted by rotations of rice with other crops, such as wheat,
90 pulse, oilseeds, maize and vegetables (Hoa et al., 2006; Goswamia et al., 2020).
91 However, the structures and patterns of microbial communities in paddy soils are
92 poorly characterized across systems, soils and crop rotation gradients in non-Asiatic
93 countries and/or temperate regions.

94 Changes in community composition, diversity and networks are most
95 determined by the crop rotation when comparing rice monoculture with rice systems
96 including legumes and other crops. Paddy soils in temperate to tropical regions of Asia
97 support bacterial communities mostly driven by soil characteristics and fungal
98 communities are better predicted by geographical distance (Jiang et al. 2016).
99 Abundance, composition, and diversity of bacterial communities are very different in
100 rotations of rice with other crops, like mungbean, wheat, or maize than rice in
101 monoculture (Jiang et al. 2016; Feng et al. 2020). Rice fertilization type also produces
102 differential effects on microbial communities, decreasing fungal richness and increasing
103 bacterial richness when organic manure is added or increasing microbial community
104 abundance under rice-wheat rotation compared with rice fallow (Chen et al. 2016; Feng
105 et al. 2020). Moreover, bacterial diversity is decreased in soils receiving straw
106 incorporation with high fertilization inputs, which denotes that higher C:N ratio is better
107 for bacterial diversity and depends on the affected bacterial phyla (Wu et al. 2020).
108 Also, in rice-wheat crop rotation with different fertilization inputs, microbial communities
109 respond differently. Bacterial community composition and functions were modified by
110 fertilization only, while fungal community composition was affected by both fertilization

111 and crop types (Chen et al. 2020). However, little is known about how different rice
112 rotations affect soil microbial communities and how it affects the productivity and
113 sustainability of these systems in temperate regions like Uruguay. Previous studies
114 showed no major differences among microbial communities associated with regards to
115 rice and pasture rotations and soils under continuous rice in South America (Fernández
116 et al., 2013; Maguire et al., 2020).

117 Diversity of microbial communities, either bacterial or fungal, inhabiting the rice
118 soil ecosystem have been characterized previously, both in anoxic bulk soil and in oxic
119 surface soil (Lee et al., 2015; Kim and Lee, 2019). Most studies reported bacterial
120 phyla *Proteobacteria*, *Chloroflexi*, *Actinobacteria* and *Acidobacteria* and fungal classes
121 *Ascomycota*, *Basidiomycota* and *Glomeromycota* dominating rice paddy soils (Jiang et
122 al., 2016). In Uruguayan conditions, *Firmicutes*, *Proteobacteria*, and *Acidobacteria*
123 were dominating soils under traditional rice and pasture rotation with high relative
124 abundance of *Firmicutes* (~40%). Other phyla were found with <10% relative
125 abundance, except for *Verrucomicrobia* and *Actinobacteria* that incremented relative
126 abundances in the soil after four years or under permanent pasture (Fernández et al.,
127 2013). Uruguayan native grassland soils of the Campos biome, where rice
128 agroecosystems are established, were dominated by *Proteobacteria*, *Actinobacteria*,
129 *Firmicutes*, *Verrucomicrobia*, *Acidobacteria*, *Planctomycetes* and *Chloroflexi* phyla
130 (Garaycochea et al., 2020).

131 Uruguayan rice system has been maintained since the 1920s in a traditional
132 rotation of rice and pastures, consisting of two years of rice and three to four years of
133 perennial pastures in legume—grass mixtures integrated with livestock production
134 (Chebataroff, 2012). Cultivation is exclusively under irrigation with a dry phase from
135 seeding to approximately one month later when it is flooded and completes the cycle
136 until harvest (Chebataroff, 2012). Rice is grown after a perennial grass and legume mix
137 pasture that can be benefited by the over 100 kg N ha⁻¹ fixed in a 26-week period
138 reported for a mixture of white clover and fescue (Labandera et al., 1988). This

139 integration of rotation systems with crops and pastures is rare globally, except in some
 140 parts of South America and particularly in Uruguay where most crop lands were
 141 historically occupied by grasslands (García-Préchac et al., 2004). This rice–pasture
 142 integration is a more sustainable system that allows preservation of natural resources,
 143 minimization of agrochemical inputs, and reduction of environmental impacts while
 144 sustaining economic productivities (Pittelkow et al., 2016). However, in recent years,
 145 there has been interest to intensify production in these systems to meet market
 146 demands and increase farmers' incomes. Intensification occurs in different ways, like
 147 augmenting the rice frequency in the rotation with new crops and/or shortening the
 148 pasture phase. Thus, new rotations are proposed and are under continuous evaluation
 149 by farmers. Nevertheless, it is unclear whether these more intensified systems are
 150 productively and environmentally sustainable over time. To answer some of the
 151 questions that arise about intensification processes, a long-term experiment (LTE) was
 152 established in 2012 in the Experimental Unit of Paso de la Laguna (UEPL), Treinta y
 153 Tres, Uruguay. The main objectives of the experiment are to evaluate and compare the
 154 effects of intensification of rice rotations in terms of productivity, inputs use, as well as
 155 environmental indicators. Information generated during the evaluation of the
 156 experiment allows the generation of guidelines for sustainable agricultural
 157 intensification for Uruguayan rice systems (Terra et al., 2014).

158 In the current study, the LTE of UEPL on rice rotations was used to test the
 159 impacts of five different rotations on bulk soil microbial communities, after a complete
 160 rotation cycle of six seasons, compared with the traditional rice and pasture system.
 161 With the hypothesis that crop rotation would have an impact on the structure of the soil
 162 bacterial and fungal community, this study analyzed which antecessor of rice had the
 163 greatest impact on soil microbial communities by comparing the different phases of the
 164 experiment and determining which species can be considered indicators for these
 165 different systems. Overall, the goal of this study was to draw a baseline of the effects of

166 crop intensification on the composition of soil microbial communities in rice fields in the
167 temperate weather of Uruguay.

168

169 **2. Materials and methods**

170 *2.1 Experimental site*

171 The study was conducted at the LTE of the UEPL established in 2012 at Treinta
172 y Tres, Uruguay (S 33° 16' 22", W 54° 10' 23"). The field experiment was an LTE for
173 the study of rice rotations with pastures and other crops like soybean and sorghum.
174 The main objectives of the LTE were to study sustainability of a medium to long time of
175 intensified rice systems in terms of productivity (Macedo et al., 2021). According to the
176 description in Onofri et al. (2016), the LTE contain several rotations of different lengths
177 and different numbers of phases per crop and rotation cycle and is maintained with
178 complete straw returning and no-tillage (Macedo et al., 2021). The experiment
179 consisted of six different rice rotations with different degrees of rice and crop
180 intensification, from a traditional Uruguayan rice–pasture system, consisting of two
181 consecutive seasons with rice and three years of a pasture with a mixture of grasses
182 and leguminous species (Deambrosi et al., 2009), to a very intensified system of
183 continuous rice (Fig. S1). Although continuous rice is a common practice in several
184 Asiatic countries, it is unpracticed in Uruguay. The six rotations are from one to six
185 years long with each phase occurring each year for each of the rotations, totaling 20
186 phases; nine of these phases are with rice each year (Fig. S1). Each phase was
187 seeded in a 20 x 60 m plot and repeated three times (blocks) for a total of 60 plots.
188 Rotations included: 1) continuous rice (CR, 1 year), 2) rice and other crops (RC, rice-
189 soybean-rice-sorghum, 4 years), 3) rice and short pasture (RSP, rice-pasture, 2 years),
190 4) traditional rice and pasture (RP, rice-rice and three years of pasture, 5 years), 5)
191 rice, soybean and pastures (RCP, rice-soybean-soybean-rice and pasture, 6 years),
192 and 6) rice and soybean (RS, rice-soybean, 2 years; Fig. S1). All rotations/phases
193 completed a full cycle in the 2017-2018 season and began a new one in the season

2018-2019 when sampling for the present study was performed before rice seeding. From the total of nine rice phases present each year, seven were selected for soil sampling (Fig. 1).

The historical mean monthly temperature in the research area is 11.5 ± 0.82 °C and 22.3 ± 0.85 °C during winter and summer respectively; mean annual rainfall is 1360 ± 315 mm; annual total potential evapotranspiration is 1138 ± 177 mm. The mean annual precipitation (2012-2018) was 1290 mm, with a monthly mean of 107 mm for the period of the experiment, from September 2012 to September 2018.

Dominant soil type in the experiment was an Argialboll with a slope less than 0.5% (Durán et al., 2006). The soil (0-15 cm) was classified as clayey silt, containing 14.2 g kg^{-1} organic carbon, 0.14% total N, 28.8 mg exchangeable Mg kg^{-1} , 659.6 mg available P kg^{-1} , and 10.5 mg exchangeable K kg^{-1} , and had a pH of 5.7 at the beginning of the experiment in September of 2012.

Management practices, fertilization, pesticide use, and irrigation were based on INIA Rice Program recommendations (Macedo et al., 2021).

2.2 Soil sampling

Soil samples were collected in September 2018 before rice seeding at the beginning of the second cycle of the experiment in seven different phases of the nine possible in the six rotations, including the two phases of the traditional rice and pasture system, before first and second rice (Fig. 1). In each 20 x 60 m plot, twenty 3-cm diameter soil cores were randomly taken from 0–10 cm depth of topsoil with a metal soil borer. The 20 soil cores were then mixed, homogenized, and separated into two samples. First, a 30-40-gram soil sample was air-dried under sterile conditions in Petri dishes, passed through a one mm metal sieve, transferred to 60 cm³ plastic jars and stored at –20 °C for DNA extraction. A second sample was air-dried and stored at 4 °C for soil chemical analysis.

222 2.3 Soil chemical analysis

223 The soil samples were analyzed for their chemical properties at the Laboratorio
224 de Suelos, INIA La Estanzuela, Uruguay. Soil samples were air-dried at room
225 temperature and passed through a one mm sieve to remove aboveground plant
226 materials, roots, and stones. The pH was measured in triplicates in the supernatant of
227 a 1:2.5 (w/v) soil:water solution ratio mixture after three minutes of shaking and 15
228 minutes of resting, using a pH meter according to Beretta et al. (2014). Available P
229 content was measured by extraction and subsequent determination of the extracted P
230 by absorbance with the Bray and Kurtz method (Bray and Kurtz, 1945). Soil organic
231 carbon was measured by dry combustion of the sample and subsequent infrared
232 detection of CO₂ with the Wright and Bailey method (Wright and Bailey, 2001).
233 Exchangeable macronutrients, such as magnesium and potassium, were measured by
234 the ammonium acetate extraction method (1 M – pH 7,0), and reading the extract was
235 completed by atomic emission (K) and atomic absorption (Mg) or by atomic emission
236 with the ICP-OES team (Jackson, 1964).

237

238 2.4 DNA extraction, amplification, Illumina NovaSeq sequencing and data analysis

239 Genomic DNA was extracted from 1g of soil from 4 different samples from each
240 sampled plot using the DNeasy PowerSoil Kit (Qiagen, Antwerp, Belgium) according
241 with the manufacturer's instructions. Concentration and quality of the DNA samples
242 was measured by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific,
243 Wilmington, DE, USA). The four samples were combined and diluted to 20 ng/μl for
244 final analysis. For bacteria, the V4–V5 region of the bacterial 16S rRNA gene was
245 amplified by PCR using the primers set 515F (5'-GTGCCAGCMGCCGCGGTAA-3')
246 and 907R (5'-CCGTCAATTCCTTTGAGTTT-3') and a high-fidelity polymerase. For
247 fungal study, the V4 region of the 18S gene was amplified using the primers set 528F
248 (5'-GCGGTAATTCCAGCTCCAA-3') and 706R (5'-AATCCRAGAATTTACCTCT-3').
249 This region of the 18S gene is more conserved than ITS or 28S ribosomal regions but

250 can be used to estimate diversity at family level. The PCR conditions were 95°C for 5
251 min, followed by 25 cycles of denaturation at 95°C for 30 s, 50°C for 30 s, 72°C for 40
252 s, and a final extension period of 72°C for 7 min.

253 The PCR products were mixed with the same volume of 2× loading buffer and
254 were subjected to 1.8% agarose gel electrophoresis for detection. Samples with a
255 bright main band of approximately 450 bp were chosen and mixed in equal-density
256 ratios. The obtained PCR products were purified using a GeneJET Gel Extraction Kit
257 (Thermo Fisher Scientific, Waltham, MA, United States). Sequencing libraries were
258 generated following manufacturer's recommendations with NEB Next® Ultra™ DNA
259 Library Prep Kit for Illumina (NEB, USA). The constructed libraries were validated using
260 an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, United States) and
261 quantified with a Qubit 2.0 Fluorometer (Thermo Fisher). Finally, paired-end
262 sequencing was conducted using an Illumina NovaSeq 6000 Sequencing System
263 platform (Illumina, Inc., San Diego CA, United States) at CD Genomics, Co., Ltd. (NY,
264 USA).

265 Paired-end reads obtained were merged using FLASH (Magoc and Salzberg,
266 2011). Quality filtering were then performed on the raw tags under specific filtering
267 conditions of Trimmomatic (Bolger et al., 2014). Assembled sequences shorter than
268 300 bp in length were eliminated from the database. UCLUST in QIIME (Caporaso et
269 al., 2010) was used to cluster the tags with 97% similarity and acquired the operational
270 taxonomic units (OTUs). Singletons were removed from the datasets as recommended
271 in previous studies (Lindahl et al. 2013). OTUs were then annotated using Silva
272 taxonomic database.

273

274 *2.5 Statistical analysis*

275 Most statistical analyses were carried out by using the Vegan package
276 (Oksanen et al., 2016) in R (version 4.0.1) (R Core Team, 2020) unless stated
277 otherwise.

278 Differences between rotations were analyzed using a one-way analysis of variance
 279 (ANOVA), and an LSD test was performed for multiple comparisons using SAS version
 280 9.4 (SAS Inc., Cary, NC, USA). Differences at $P < 0.05$ level were considered
 281 significant. The data reported for microbial diversity are mean values from the three
 282 replicates (plots) analyzed. Rarefaction curves and observed species were calculated
 283 using the Vegan package in R (Oksanen et al. 2019). The relative abundance-based
 284 coverage estimator (ACE), Chao1 lower-bound estimator of species richness (Chao1)
 285 richness index, and Shannon entropy (H') and Simpson (S) diversity index were
 286 generated with estimateR function from the Vegan package in R (Oksanen et al. 2019).
 287 Alpha-diversity estimates for each diversity index between rice rotations were tested by
 288 one-way ANOVA and post-hoc Tukey's HSD to identify significant differences among
 289 group means ($P < 0.05$). Non-metrical multidimensional scaling (NMDS) was run for
 290 comparison of beta-diversity measures on the Hellinger-transformed OTU tables,
 291 separately for bacterial and fungal communities. Ordination was run with parameters
 292 set on Bray-Curtis distance measure and two dimensions and total stress obtained in
 293 the Vegan package in R (Oksanen et al. 2019). Permutational multivariate analysis of
 294 variance (PERMANOVA, Anderson 2001) was used separately to determine the effect
 295 of the studied rotation systems compared with the traditional rice and pasture on the
 296 observed variance of the total bacterial and fungal communities. The bacterial and
 297 fungal communities were first tested for heteroscedasticity using betadisper function (P
 298 < 0.05) and permutational multivariate analysis of variance using adonis function in the
 299 Vegan package in R (Oksanen et al. 2019) on Bray-Curtis distance matrices obtained
 300 from OTU tables. Additionally, when a significant treatment effect was found a post hoc
 301 pairwise comparison was performed. Relative abundance of bacterial or fungal OTUs
 302 was analyzed for correlations to soil chemical properties using a canonical
 303 correspondence analysis (CCA; ter Braak, 1986) in R using Vegan and Spearman's
 304 correlation coefficients. Analysis was also performed to assess possible relationships
 305 between relatively abundant bacterial and fungal phyla and soil chemical properties.

Bacterial and fungal taxa that characterize microbial communities in the different rice rotation soils studied were identified using linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al., 2011). The classified taxa were discriminated accordingly in relation to having both high relative abundances within sampling soils and high frequencies across replicated plots within each rice rotation. LEfSe method identifies bacterial and fungal biomarkers via Kruskal-Wallis non-parametric test detecting significant taxa and performing an LDA effect size estimation. Biomarker taxa were identified with an $\alpha = 0.05$ and an effect size threshold of LDA = 2. Biomarker taxa were then plotted across each rice rotation to help the visualization of the respective frequencies and relative abundances of occurrence (Segata et al., 2011). LEfSe analysis was performed through the Galaxy/Hutlab server (huttenhower.sph.harvard.edu/galaxy).

318

3. Results

3.1 Soil physico-chemical analysis

Among the chemical parameters analyzed, P and the relation C:N showed significant statistical differences between rice rotations (Table 1). Phosphorus concentration ($P = 0.0162$) was lower in continuous rice and the rotations with pastures in the system and higher in rotations of rice with crops (RC and RS). The relation C:N ($P = 0.0415$) was lower in the rotations with more time occupied with crops, rice, soybean, and/or sorghum in the sequence (Table 1). No statistical differences were found for K, Mg, organic C, N, organic matter, or pH among rotations after six years of the experiment (Table 1).

329

3.2 Sequencing information

A total of 1,557,096 paired-end sequences were obtained for 16S and 1,778,576 for 18S. Bacterial paired-end reads were overlapped to obtain high-quality tag sequences and after filtering 658,727 effective tag sequences were obtained with average lengths

of 373 bp. After filtering, and discarding non-fungal tags, paired-end reads for 18S accounted for 647,806 effective tag sequences with an average length of 307 bp. OTU clusters were defined by a 97% identity threshold and 3662 and 807 OTUs were found for the bacterial and fungal communities, respectively.

3.3 Diversity and richness of the soil community

Obtained rarefaction curves showed that archaeal/bacterial communities were better represented than fungal communities (Fig. S2, Fig. S3). However, similar patterns were obtained for sequenced samples within both microbial groups from all soils suggesting that similar levels of diversity values were captured for each soil sampled (Fig. S2, Fig. S3).

Alpha diversity of the microbial communities in the soils studied was assessed by means of different richness and diversity indices. The observed number of bacterial OTUs was statistically different between rotations ($F = 3.9577$, $P = 0.0159$) with CR having the highest OTU number and the rotation RP2 and the agricultural rotations, RC and RS, having the lowest OTU numbers. Shannon index was statistically significant ($F = 2.9044$, $P = 0.0470$) with continuous rice different from the other rotations, except for RCP (Table 2). No significant differences were found in the richness indices, Chao1 and ACE, and Simpson diversity index among the different rotations studied (Table 2).

Fungal OTU numbers were higher in rotation RP2 and at lower OTU number was found in RCP. Shannon diversity index varied between rotations ($F = 3.0342$, $P = 0.0408$), with the highest value in rotations RS and RP2 and lowest value in CR and RP (Table 3). No significant differences for ACE and Chao1 richness indices and Simpson diversity index ($P = 0.07$) for fungal communities were observed among rice rotation soils (Table 3).

3.4 Archaeal, bacterial and fungal community composition

361 The prokaryote community was represented at most relative abundance by
362 domain Bacteria (98.9%) not Archaea (1.02%). Whole bacterial community composition
363 within the different soils studied showed *Proteobacteria*, *Firmicutes*, *Chloroflexi*,
364 *Actinobacteria*, *Planctomycetes* and *Acidobacteria* as the most OTU rich at the phylum
365 level (Fig. S4). The bacterial phyla with the highest relative abundance in the whole
366 study were *Actinobacteria* (35.8%), *Firmicutes* (19.9%) and *Proteobacteria* (17.4%).
367 The remaining important phyla (*Acidobacteria*, *Chloroflexi*, *Verrucomicrobia*,
368 *Planctomycetes* and others) accounted for less than 10% but more than 1% of the
369 abundance each. Sequences obtained for each rotation were associated with nine
370 bacterial phyla with >1% of relative abundance in at least one rotation or 18 phyla with
371 >0.1% of relative abundance (Fig. 2). *Actinobacteria*, *Firmicutes*, and *Proteobacteria*
372 ranged from 32.5–39.6%, 18.3–22.4%, and 16–19.1% relative abundance,
373 respectively, according to the rotation (Fig. 2). *Chloroflexi*, *Acidobacteria*,
374 *Verrucomicrobia* and *Planctomycetes* were found at relative abundances <10% but
375 >1%. Relative abundances >1% were found for *Euryarchaeota* (Archaea) and
376 *Bacteroidetes* only in CR and in CR and RSP, respectively (Fig. 2). Relative
377 abundance of *Actinobacteria* did not differ between the seven rotations studied ($F =$
378 1.0003, $P = 0.4626$), but the lowest relative abundances were found in the rotations
379 with 100% of agriculture of rice with other crops (RC and RS). Continuous rice
380 incremented the abundance of *Proteobacteria* ($F = 3.6504$, $P = 0.0215$), and the
381 relative abundance was significantly higher than in the rotations including other crops
382 like soybean and sorghum (rotations RC, RCP and RS), but not different from rotations
383 with more pastures in the cycle (Fig. 3). *Chloroflexi* was incremented in treatments with
384 more crops in the cycle, but differences were not statistically significant ($F = 2.1706$, P
385 $= 0.1089$). Comparing the rotations by pairs showed CR differed statistically from the
386 less intensive rotations of rice with longer pastures cycles (rotations RP, RP2 and
387 RCP). *Verrucomicrobia* differed statistically between rotations ($F = 3.7177$, $P = 0.0201$)
388 with higher relative abundances in the rotations of RS and second rice with long

pastures and RC (Fig. 3). Other less represented phyla of bacteria or archaea were statistically different within treatments. *Crenarchaeota* (Archaea, $F = 15.1422$, $P = 0.0001$) and *Nitrospirae* ($F = 3.5084$, $P = 0.0248$) were more abundant in continuous rice than other rotations, while *Cyanobacteria* ($F = 4.6344$, $P = 0.0085$) was more abundant in continuous rice than other rotations, except for RSP, and the rotations with other crops had the lowest relative values (Fig. S5).

Fungal community composition in the soils from different rotations showed that most OTUs (388) remain unidentified (Fig. S4). *Chytridiomycota* (93), *Ascomycota* (84), *Cryptomycota* (58), *Basidiomycota*, (40) and *Aphelidea* (39) were the most OTU rich phyla in the kingdom Fungi (Fig. S4). The highest relative abundance was found for *Ascomycota* (68.4%), ranging from 65.5% in RS rotation to 71.8% in RP2 (Fig. 2). *Basidiomycota* (2.71%), *Mucoromycota* (2.57%), and *Mortierellomycota* (0.987%), were fungal taxa accounting for relative abundances around 1% or more. The phyla, *Aphelidea*, *Cryptomycota*, *Mucoromycota*, *Neocallimastigomycota* and *Incertae sedis* (Fungi) were represented by relative abundances of <1% but over 0.1%. Other taxa were represented but with very low relative abundances (<0.1%, including *Blastocladiomycota*, *Glomeromycota*, *Nucleariida*, *Olpidiomycota*, and *Zoopagomycota*). Some of these phyla varied significantly among rotations, including *Cryptomycota* ($F = 3.1742$, $P = 0.0351$), *Glomeromycota* ($F = 2.9461$, $P = 0.0449$), and *Incertae sedis* ($F = 3.8275$, $P = 0.0181$) (Fig. 4). Within the *Ascomycota*, the most abundant fungal phyla, *Sordariomycetes*, *Eurotiomycetes* and *Dothideomycetes*, accounted for >95% of the relative abundance. Orders with the highest relative abundances were *Hypocreales* (30.8%), *Eurotiales* (25.5%), *Pleosporales* (19%), *Sordariales* (16.3%), *Capnodiales* (2.4%) and *Boliniales* (1.3%). Others, unidentified (2.3%), and *Incertae sedis* (2.4%) completed the list of fungal taxa found. *Basidiomycota*, *Tremellales* (57.8%), *Atheliales* (20.7%), *Ceratobasidiaceae* (5.9%), *Cystofilobasidiaceae* (3.9%) and *Sporidiobolaceae* (2.9%) were the most

abundant taxa within the phylum. *Tremellales* was represented exclusively by *Saitozyma* spp. and *Atheliales* exclusively by *Athelia rolfsii* (Table S2). Four fungal taxa (*Fusarium oxysporum*, unidentified *Aspergillaceae*, *Chaetomium globosum* and unidentified Fungi) accounted for >50% of the relative abundance observed (Table S2).

3.5 Microbial community composition

Non-metric multidimensional scaling (NMDS) ordination constructed on total bacterial OTU composition showed that samples clustered in three groups consistent with the percentage of years occupied with rice in the rotation cycle. One group formed alone with rotation CR (100% rice); a second group formed with the cluster of rotations with other crops or short pasture (RC, RS, and RSP with 50% of time occupied with rice); and a third group formed with more lax rice rotations, including traditional rice and pastures (RP, RP2, and RCP 33–40% of time occupied with rice, Fig. 5). NMDS ordination for archaeal/bacterial communities resulted in a two-dimensional final resolution with a stress value of 0.196 (Fig. 5). PERMANOVA indicated a significant effect of the rotation system upon the bacterial OTU assemblages ($F = 1.548$, $P = 0.0012$). Permutation test for homogeneity of multivariate dispersions showed a nonsignificant dispersion ($F = 0.223$, $P = 0.963$). However, post hoc analysis failed to discriminate differences between pairs of soils ($P < 0.05$).

The NMDS analysis of fungal communities showed a separation of communities according to rotation, but without grouping within different soils. The analysis showed a separation into three groups, mostly according to the previous crop/pasture as a rice antecessor. These groups consisted of two rotations with rice as antecessor (CR and RP2), two rotations with soybean/sorghum as antecessors (RC and RS) and a third group with one to three years of pastures as antecessors (RP, RSP and RCP, Fig. 6). The NMDS ordination of fungal communities also resulted in a two-dimensional final resolution with a stress value of 0.16 (Fig. 6). PERMANOVA indicated a significant

effect of the rotation system upon the fungal OTU assemblages ($F = 1.635$, $P = 0.0051$).
Permutation test for homogeneity of multivariate dispersions showed a nonsignificant
dispersion ($F = 0.587$, $P = 0.736$). However, post hoc analysis failed to discriminate
differences between pairs of soils ($P < 0.05$).

Canonical correspondence analysis (CCA) was used to determine the
correlation of soil chemical results on individual archaeal/bacterial and fungal OTUs
(Fig. 7, Fig. 8). Individual bacterial OTUs appear to be weakly influenced by P, pH, and
Mg and fungal OTUs appear to be weakly influenced by P, as shown by the OTUs
dispersion around the vectors representing chemical values on the CCA (Fig. 7, Fig. 8).
Additionally, rho values of Spearman correlations of the most abundant ($> 0.01\%$)
bacterial phyla with soil chemical parameters were analyzed. Seven of the bacterial
phyla (*Actinobacteria* (K), *Proteobacteria* (P), *Verrucomicrobia* (P), *Synergistetes* (C
and N), *Thaumarchaeota* (pH), *Cyanobacteria* (K) and *Nitrospirae* (C/N)) significantly
($P < 0.05$) correlated with at least one of these chemical parameters. Also, nine phyla
(*Actinobacteria* (P), *Proteobacteria* (K), *Verrucomicrobia* (K), *Synergistetes* (K),
Thaumarchaeota (P), *Planctomycetes* (K), *Chloroflexi* (C:N), *Spirochaetes* (C and N)
and *Gemmatimonadetes* (P)) were weakly ($P < 0.10$) correlated with at least one
chemical parameter (Table S3). Within fungal phyla, rho values of Spearman
correlations of fungal classes showed that five (*Ascomycota* (K and Mg),
Chytridiomycota (P), *Glomeromycota* (P), *Nucleariida* (P) and *Zoopagomycota* (P))
were correlated ($P < 0.05$) with one or two chemical parameters evaluated. Also, five
fungal phyla (*Aphelidea* (N, pH), *Chytridiomycota* (Mg), *Cryptomycota* (C/N),
Glomeromycota (pH), *Neocallimastigomycota* (pH)) were weakly ($P < 0.10$) correlated
with at least one chemical parameter (Table S3).

Taxonomic biomarkers for archaea/bacteria and fungi among rice rotations
were detected via Linear discriminant analysis Effect Size (LEfSe). The LEfSe analysis
for bacterial communities revealed that there were more biomarkers with larger effect
sizes (LDA score > 2.5) associated with the CR rotation, while agricultural rotations

showed the lowest number of bacterial biomarkers (Fig. 9, Fig. S6). Rotations including pastures showed intermediate numbers of biomarker taxa (LDA score > 2.5). Considering the traditional rice and pasture rotation, prokaryotic indicator taxa that contributed the most were *Acetobacteraceae* and *Promicromonosporaceae* after pasture (RP), while *Betaproteobacteriales* and *Pseudonocardia* characterized the rotation after rice (RP2) (Fig. 9, Fig. S6). Other rotations after pasture that showed intermediate numbers of biomarkers (LDA score > 2.5) include *Pseudonocardiales* and *Actinomycetospora* as the most characteristic for RCP and *Ruminococcaceae*, *Paenibacillaceae* and *Ammoniphilus* as the most characteristic for RSP (Fig. 9, Fig. S6). Biomarker taxa for CR included Bacteria *Anaerolineae*, *Methylocystis*, *Nakamurella*, *Mycobacterium*, uncultured *Chloroflexi*, and *Phenylobacterium* and Archaea *Euryarchaeota*, *Bathyarchaeia*, *Chrenarchaeota* and *Methanosarcina* (Fig. 9, Fig. S6). More agricultural rotations were characterized by *Ktedonobacterales* for RC and *Rokubacteriales* for RS (Fig. 9, Fig. S6). Fungi contributed less biomarkers for rotation characterization and only eight taxa were found with significant values (LDA score > 2.5) for four rotations, CR, RP, RP2, and RS. Interestingly, rice and pasture, after pasture or rice (RP and RP2), were characterized by five biomarker taxa, but these were unidentifiable at the taxonomic level, except for an *Acremonium* sp. (Fig. S7).

4. Discussion

Metagenome study results based on NovaSeq sequencing from the first rotational cycle of the LTE demonstrated that differences in crops and management practices shaped soil microbial communities after six years, although at different degrees depending on the group analyzed.

Physicochemical analysis show that only P and C:N varied among the rotations studied (Table 1). Phosphorous concentration was higher in rotations of rice associated with other crops, like soybean and sorghum, due to the residual P from the previous

500 fertilization. The C:N was higher in rotations associated with pastures previous to rice
501 being one to three years long in the rotation due to the incorporation of carbon by the
502 residues from the pastures. No clear correlations were obtained between different
503 groups of prokaryotes and fungi and the soil parameters, except for some such as P
504 and C:N (Table S3), and this could be due to the few changes occurring in the soils
505 after six years of the experiment.

506 Differences among bacterial and fungal richness were found between rotations
507 studied; the highest bacterial diversity and lowest fungal diversity was found in CR
508 (Table 2 and 3). The high bacterial diversity in CR is contrary to reports indicating a
509 decrease in diversity of microorganisms in simplified rotations of agricultural systems
510 (Xuan et al. 2012; Venter et al. 2016). However, except for the OTU number no
511 statistical differences in abundance and diversity index were found between rotations
512 after six years (Table 1). The most abundant bacterial phyla were *Actinobacteria*,
513 *Firmicutes*, *Proteobacteria*, and to a lesser extent (< 10% relative abundance)
514 *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia* and *Planctomycetes*. These dominant
515 bacterial phyla were previously reported as ubiquitous in worldwide soils (Delgado-
516 Baquerizo et al. 2018). Although *Actinobacteria* and *Firmicutes* are not commonly
517 reported as the most abundant in rice soils, these were the most abundant phyla found,
518 respectively, in experiments of preincubated and stored drained and flooded paddy
519 soils (Wang et al. 2015). *Actinobacteria* sequences ranged from 32.5% (RS) to 39.6%
520 (RCP) of relative abundance, quite higher than previous reports for this group in paddy
521 soils, which ranged from 10 to 16% (Fernández et al. 2013; Jiang et al. 2016; Chen et
522 al. 2016; Ji et al. 2020) or as low as <3% (Xuan et al. 2012) but slightly above the world
523 relative abundance reported previously for this group (>30%, Delgado-Baquerizo et al.
524 2018). *Actinobacteria* abundances were higher in rotations with pasture and lower with
525 crops, but without statistical differences, perhaps because only one cycle was
526 completed, and more years are needed to differentiate the communities. Results are
527 consistent with Lauber et al. (2008) who found more abundant *Actinobacteria*, also not

significantly, in pasture soils, and Araujo et al. (2020) who reported reduced actinobacterial diversity with agriculture-based disturbance. *Actinobacteria* are Gram positive, aerobic, saprophytic bacteria reported across a diverse range of ecologies and environments, with higher abundances in warm dry soils due to its ability to tolerate drought (Delgado-Baquerizo et al. 2018; Ren et al. 2018; Araujo et al. 2020). Some members are known for their ability to secrete diverse types of metabolites such as antibiotics and extracellular enzymes (Selvakumar et al. 2014). The relatively high abundance of *Actinobacteria* found could be influenced by the timing of the soil sampling before rice sowing. The stress imposed during the fallow time after the use of herbicides in the previous pasture or cover crop established conditions of dry soil with a high contribution of organic residues that favored this group (Selvakumar et al. 2014; Araujo et al. 2020). *Firmicutes* was the bacterial phylum with the second highest relative abundance (19.9%) but without differences between rotations. The abundance is not surprising since it was the most abundant phylum found in a previous study from rice and pastures soil (28-40%, Fernández et al. 2013) and grasslands (17%, Garaycochea et al. 2020) in Uruguay. Data from paddy soils indicate that *Firmicutes* was represented by low percentages (<10%) in most situations (Xuan et al. 2012; Jiang et al. 2016; Ji et al. 2020). Also, the two most common, and four of the twenty most common bacterial OTU were *Firmicutes* members, mainly *Bacillales*, accounting for >10% of the total abundance (Table S1). *Firmicutes* is the most important group fermenting organic matter in anoxic conditions and has the ability to form endospores adapting to the stress of the dry conditions imposed by the fallow in the transitions of the pasture phase of the rotation (Fernández et al. 2013). *Proteobacteria* accounted for 17.4% of the sequences, ranging from 16% in rotations with crops (RC and RS) to 19% (RP2). These relative abundances are quite lower than world reported mean (>35%, Delgado-Baquerizo et al. 2018), and slightly lower than values reported in rice fields in Uruguay (20-28%, Fernández et al. 2013) and worldwide (26-38%, Jiang et al. 2016; Chen et al. 2016; Zhao et al. 2017). The abundance of *Proteobacteria* decreased

556 according to the type of crop in the rotation and not according to the level of
557 intensification. Higher values were found in rice with pastures (RP, RP2 and RSP) and
558 CR and lower in rotations with other crops such as soybean and sorghum (RC, RCP,
559 RS). Furthermore, *Proteobacteria* was the main or second most important phyla found
560 in prokaryotic communities of Uruguayan soils in grassland and rice-pasture rotation
561 (Fernández et al. 2013; Garaycochea et al. 2020). Members of *Proteobacteria* play
562 important roles such as P solubilization, N fixation, plant growth promotion, and
563 indication of copiotrophic soils, suggesting a better nutrient status and associated with
564 higher rice yields (Huang et al. 2020).

565 Fungal communities were represented mainly by unidentified taxa (388 OTUs)
566 that show the complexity of this group of organisms and denote the limited information
567 available on the 18S region. More studies are needed that allow a more efficient
568 identification at a lower taxa level, for example, by sequencing other regions such as
569 ITS. Within the identified groups, *Ascomycota* dominated in all the soils in accordance
570 with the fact that this is the most abundant group of fungi on a world scale with an
571 average >68% relative abundance (Egidi et al. 2019). *Sordariomycetes* was the most
572 abundant class represented, which includes *Pyricularia oryzae* (causal agent of rice
573 blast) and *Nakataea oryzae* (causal agent of stem rot of rice), the two most devastating
574 pathogens of rice in Uruguay (Martínez 2016; Martínez et al. 2018). *Hypocreales* was
575 the most represented order of fungi and this was mostly represented by *Bionectriaceae*
576 and *Nectriaceae* species, such as *Fusarium oxysporum* (Table S2). *Basidiomycota* and
577 *Mucoromycota* were the second most abundant phyla with similar abundances (2.71%
578 and 2.57% respectively). *Basidiomycota* was most represented in agricultural rotations
579 (RC and RS, Fig. 2) with most sequences assigned to *Athelia rolfsii* (Table S2) a
580 common pathogen of soybean in Uruguay (Stewart and Rodríguez, 2016).
581 *Mucoromycotina* was highly represented only in one of the samples in the RP rotation
582 associated with a high sequence count of the saprophytic *Rhizopus oryzae* (Table S2).
583 Only *Cryptomycota*, *Glomeromycota* and *Incertae sedis* had differences in abundance

584 between rice rotations (Fig. 4). Although *Glomeromycota* (also known as arbuscular
585 mycorrhizal fungi or AMF) abundances were very low, higher values were found in
586 rotations with soybean (RS and RC, Fig. 4), as reported in the same experiment by
587 Rodríguez-Blanco and Giménez (2019) who found that roots of soybean plants were
588 heavily colonized by *Glomeromycota* communities. However, more studies are needed
589 on the relationship of *Glomeromycota* with the rice and pastures rotations (RP and
590 RP2) since no differences in abundances were found with soybean rotations (Fig. 4).

591 These results demonstrated the responses of the bacterial/archaea and fungal
592 communities to crop rotation and intensification despite the similarity between some of
593 the results and the fact that only one cycle elapsed. Comparing the total OTUs of the
594 bacterial communities showed that the rotations were clustered into three groups. The
595 lax rotations with 33–40% of time with rice (with two to three years of pasture in the
596 rotation), including the traditional rice and pasture rotation phases (RP and RP2) and
597 RCP, cluster in one group. A second group consists of rotations that include crops and
598 short pasture (RS, RC, and RSP, with 50% of time with rice), and a third group
599 consisting of continuous rice (CR, with 100% time with rice) in an isolated position (Fig.
600 5). These results showed that rice rather than soil use intensity or cover crop shaped
601 the soil bacterial community after a complete cycle. Physicochemical changes that are
602 occurring in the soil due to different uses are conditioning these microbial communities.
603 Fungal communities are a little different and cluster mostly according to previous
604 crop/pasture present in the rotation, although differences are poorly delineated after six
605 years. Again, a greater definition in the identification of fungal taxa by sequencing other
606 regions can help to better delineate these communities. The three groups clustered
607 according to rice, pasture (one to three years long), or crops (soybean/sorghum) as
608 antecessors (Fig. 6). This result is consistent with several studies that point out that
609 plant type affect soil fungal community composition (Schmidt et al., 2019). Fungi
610 depends more on plant products and their biotrophic interactions with different plant
611 species because a large proportion of fungi interact narrowly as pathogens,

saprotrophs or mutualists of plants (Millard and Singh, 2010; Schmidt et al., 2019). A more complete study, better delineating the taxa and making associations at the trophic or guild level of the fungi, is desirable to better characterize the communities and relationships existing in this group according to the rotation studied.

Some taxa were identified as biomarkers by LEfSe analysis for some of the rotations studied. While in the lax rotations (RP and RP2) *Proteobacteria* and *Actinobacteria* are the associated biomarkers, in intensive rotations (CR) there is a greater diversity of biomarkers, including *Actinobacteria*, *Proteobacteria* and *Chloroflexi* and Archaea. Archaeal communities including methanogenic *Methanosarcinaceae* are distinctive in irrigated rice field soils from most geographic regions (Fernández et al. 2013). Biomarkers found for RSP were mostly *Firmicutes* (*Ruminococcaceae*, *Paenibacillaceae* and *Ammoniphilus*), but reasons for this finding are unknown at this moment. Garaycochea et al. (2020) reported a higher abundance of *Firmicutes* in grassland soils with higher water holding capacity and permanent wilt point. LEfSe analysis for fungi failed to discriminate biomarkers and a more detailed study is needed to determine fungal indicator taxa for the rotations studied.

628

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635 **Bibliography**

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32-46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>.

639

640 Araujo, R., Gupta, V.V.S.R., Reith, F., Bissett, A., Mele, P., Franco, C. M. M., 2020.
641 Biogeography and emerging significance of Actinobacteria in Australia and Northern
642 Antarctica soils. *Soil Biology and Biochemistry* 146, 107805.
643 <https://doi.org/10.1016/j.soilbio.2020.107805>.
644
645 Bardgett, R., van der Putten, W., 2014. Belowground biodiversity and ecosystem
646 functioning. *Nature* 515, 505–511. <https://doi.org/10.1038/nature13855>.
647
648 Bending, G.D., Turner, M.K., Rayns, F., Marx, M.C., Wood, M., 2004. Microbial and
649 biochemical soil quality indicators and their potential for differentiating areas under
650 contrasting agricultural management regimes. *Soil Biology and Biochemistry* 36, 1785-
651 1792. <http://dx.doi.org/10.1016/j.soilbio.2004.04.035>
652
653 Beretta, A., Baddahun, D., Musselli, R., 2014. ¿Medir el pH del suelo en reposo o
654 agitando la mezcla suelo:agua?. *Agrociencia (Uruguay)* 18(2), 90 - 94.
655
656 Bolger, A. M., Lohse, M., Usadel, B., 2014. Trimmomatic: A flexible trimmer for Illumina
657 Sequence Data. *Bioinformatics* 30, 2114-2120.
658 <https://doi.org/10.1093/bioinformatics/btu170>
659
660 Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of
661 phosphorus in soils. *Soil Science* 59, 39-45.
662
663 Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor
664 influencing microbial community structure and enzyme activities across seven
665 biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry* 44, 9–20.
666 <https://doi.org/10.1016/j.soilbio.2011.09.003>.
667

668 Brune, A., Frenzel, P., Cypionka, H., 2000. Life at the oxic-anoxic interface:
669 microbial activities and adaptations. FEMS Microbiology Reviews 24, 691–710.
670 [https://doi.org/10.1016/S0168-6445\(00\)00054-1](https://doi.org/10.1016/S0168-6445(00)00054-1)KP004460.
671
672 Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., Deyn, G. D., de Goede, R.,
673 Fleskens, L., Geissen, V., Kuyper, R. W., Mäder, P., Pulleman, M., Sukkel, W., van
674 Groenigen, J. W., Brussaard, L., 2018. Soil quality – a critical review. Soil Biology and
675 Biochemistry 120, 105-125. <https://doi.org/10.1016/j.soilbio.2018.01.030>.
676
677 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello,
678 E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T.,
679 Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D.,
680 Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J.,
681 Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-
682 throughput community sequencing data. Nature Methods 7, 335–336.
683
684 Chebataroff, N., 2012. Arroz uruguayo. Editorial Agropecuaria Hemisferio Sur,
685 Montevideo, pp. 352.
686
687 Deambrosi, E., Montossi, F., Saravia, H., Blanco, P., Ayala, W., 2009. 10 años de la
688 Unidad de Producción Arroz- Ganadería. INIA Serie Técnica Nº 180, pp. 1-209.
689
690 Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge,
691 D.J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018. A global atlas of the
692 bacteria found in soil. Science 359, 320–325. <https://doi.org/10.1126/science.aap9516>.
693

694 Duran, A., Califra, A., Molfino, J.H., Lynn, W., 2006. Keys to Soil Taxonomy for
695 Uruguay. US Department of Agriculture, Natural Resources Conservation Service,
696 Washington, DC.
697
698 Egidi, E., Delgado-Baquerizo, M., Plett, J. M., Wang, J., Eldridge, D. J., Bardgett, R. D.,
699 Maestre, F. T., Singh, B. K., 2019. A few Ascomycota taxa dominate soil fungal
700 communities worldwide. *Nature Communications* 10, 2369.
701 <https://doi.org/10.1038/s41467-019-10373-z>
702
703 Feng, B., Chen, S., Chu, G., Chen, T., Fu, G., Wang, D., Xu, C., Tao, L., Zhang, X.,
704 2020. Effects of crop rotation systems on microbial structure under low N application in
705 rice field. *Journal of Plant Nutrition* 43(4), 500-511.
706 <https://doi.org/10.1080/01904167.2019.1685104>
707
708 Fernández, A., Ji, Y., Pump, J., Klose, M., Claus, P. y Conrad, R., 2013. Structure and
709 function of the methanogenic microbial communities in Uruguayan soils shifted
710 between pasture and irrigated rice fields. *Environmental Microbiology* 15(9), 2588-
711 2602. <https://doi.org/10.1111/1462-2920.12161>
712
713 Garaycochea, S., Romero, H., Beyhaut, E., Neal, A. L., Altier, N., 2020. Soil structure,
714 nutrient status and water holding capacity shape Uruguayan grassland prokaryotic
715 communities. *FEMS Microbiology Ecology* 96(12), fiae207.
716 <https://doi.org/10.1093/femsec/fiae207>
717
718 García-Prèchac, F., Ernst, O., Siri-Prieto, G., Terra, J.A., 2004. Integrating no-till into
719 Crop-pasture rotations in Uruguay. *Soil and Tillage Research* 77(1), 1-13.
720 <https://doi.org/10.1016/j.still.2003.12.002>.
721

722 Geisseler, D., Scow, K. M., 2014. Long-term effects of mineral fertilizers on soil
723 microorganisms - a review. *Soil Biology and Biochemistry* 75, 54-63.
724 <http://doi.org/10.1016/j.soilbio.2014.03.023>
725
726 Goswamia, S. B., Mondala, R., Mandib, S. K., 2020. Crop residue management options
727 in rice–rice system: a review. *Archives of Agronomy and Soil Science* 66, 9 1218–
728 1234. <https://doi.org/10.1080/03650340.2019.1661994>
729
730 Hoa, N. M., Janssen, B. H., Oenema, O., Dobermann, A., 2006. Comparison of partial
731 and complete soil K budgets under intensive rice cropping in the Mekong Delta,
732 Vietnam. *Agriculture, Ecosystems & Environment* 116 (1-2), 121-131.
733 <https://doi.org/10.1016/j.agee.2006.03.020>
734
735 Huang, M., Tian, A., Chen, J., Cao, F., Chen, Y., Liu, L., 2020. Soil bacterial
736 communities in three rice-based cropping systems differing in productivity. *Scientific*
737 *Report* 10, 9867. <https://doi.org/10.1038/s41598-020-66924-8>.
738
739 IRRI, 2006. *Bringing Hope, Improving Lives: Strategic Plan 2007–2015*, Manila. 61 p.
740
741 Jackson, M.L., 1964. *Análisis químico de suelos*. Ediciones Omega S.A. Barcelona.
742 662p.
743
744 Ji, Y., Conrad, R., Xu, H., 2020. Responses of archaeal, bacterial, and functional
745 microbial communities to growth season and nitrogen fertilization in rice fields. *Biology*
746 *and Fertility of Soils* 56,81–95. <https://doi.org/10.1007/s00374-019-01404-4>.
747
748 Jiang, Y. J., Liang, Y. T., Li, C. M., Wang, F., Sui, Y. Y., Suvannang, N., Zhou, J. Z.,
749 Sun, B., 2016. Crop rotations alter bacterial and fungal diversity in paddy soils across

750 East Asia. *Soil Biology and Biochemistry* 95, 250-261.

751 <https://doi.org/10.1016/j.soilbio.2016.01.00>

752

753 Kim, H., Lee, Y. H., 2019. The Rice Microbiome: A model platform for crop holobiome.

754 *Phytobiomes Journal* 4(1), 5-18. <https://doi.org/10.1094/PBIOMES-07-19-0035-RVW>

755

756 Labandera, C., Danso, S.K.A., Pastorini, D., Curbelo, S., Martin, V., 1988. Nitrogen

757 fixation in a white clover-fescue pasture using three methods of Nitrogen-15 application

758 and residual Nitrogen-15 uptake. *Agronomy Journal* 80(2), 265-268.

759 <https://doi.org/10.2134/agronj1988.00021962008000020022x>.

760

761 Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil

762 properties on the structure of bacterial and fungal communities across land-use types.

763 *Soil Biology and Biochemistry* 40, 2407-2415.

764 <https://doi.org/10.1016/j.soilbio.2008.05.021>.

765

766 Lee, H. J., Jeong, S. E., Kim, P. J., Madsen, E. L., and Jeon, C. O., 2015.

767 High resolution depth distribution of Bacteria, Archaea, methanotrophs, and

768 methanogens in the bulk and rhizosphere soils of a flooded rice paddy. *Frontiers on*

769 *Microbiology* 6, 639. <https://doi.org/10.3389/fmicb.2015.00639>

770

771 Macedo, I., Terra, J.A., Siri-Prieto, G., Velazco, J.I., Carrasco-Letelier, L., 2021. Rice-

772 pasture agroecosystem intensification affects energy use efficiency. *Journal of Cleaner*

773 *Production* 278, 123771. <https://doi.org/10.1016/j.jclepro.2020.123771>.

774

775 Magoc, T., Salzberg, S., 2011. FLASH: Fast length adjustment of short reads to

776 improve genome assemblies. *Bioinformatics* 27, 2957-2963.

777

778 Maguire, V. G., Bordenave, C. D., Nieva, A. S., Llamas, M. E., Colavolpe, M. B., Gárriz,
779 A., Ruiz, O. A., 2020. Soil bacterial and fungal community structure of a rice
780 monoculture and rice-pasture rotation systems. *Applied Soil Ecology* 151, 103535.
781 <https://doi.org/10.1016/j.apsoil.2020.103535>.
782
783 Martínez, S., 2016. Effects of combined application of potassium phosphite and
784 fungicide on stem and sheath disease control, yield, and quality of rice. *Crop Protection*
785 89, 259-264. <https://doi.org/10.1016/j.cropro.2016.08.002>.
786
787 Martínez, S., Bao, L., Escalante, F., 2018. Manual de identificación de enfermedades y
788 plagas en el cultivo de arroz. Boletín de Divulgación N°116. Montevideo, Uruguay,
789 INIA. <http://www.inia.uy/Publicaciones/Paginas/publicacionAINFO-58001.aspx>.
790
791 McGuire, K.L., Treseder, K.K., 2010. Microbial communities and their relevance for
792 ecosystem models: Decomposition as a case study. *Soil Biology and Biochemistry*
793 42(4), 529-535. <https://doi.org/10.1016/j.soilbio.2009.11.016>.
794
795 Onofri, A., Seddaiu, G., Piepho, H.P., 2016. Long-Term Experiments with cropping
796 systems: Case studies on data analysis. *European Journal of Agronomy* 77, 223-235.
797 <https://doi.org/10.1016/j.eja.2016.02.005>
798
799 Ren, C., Chen, J., Lu, X., Doughty, R., Zhao, F., Zhong, Z., Han, X., Yang, G., Feng,
800 Y., Ren, G., 2018. Responses of soil total microbial biomass and community
801 compositions to rainfall reductions. *Soil Biology and Biochemistry* 116, 4-10.
802 <https://doi.org/10.1016/j.soilbio.2017.09.028>
803

804 Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S.,
805 Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. *Genome*
806 *Biology* 12, 1–18. <https://doi.org/10.1186/gb-2011-12-6-r60>.
807
808 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al.
809 (2016). *vegan: Community Ecology Package*. R Package Version 2.4-2.
810
811 Pittelkow, C. M., Zorrilla, G., Terra, J., Riccetto, S., Macedo, I., Bonilla, C., Roel, A.,
812 2016. Sustainability of rice intensification in Uruguay from 1993 to 2013. *Global Food*
813 *Security* 9, 10-18. <https://doi.org/10.1016/j.gfs.2016.05.003>.
814
815 R Core Team (2020). *R: A Language and Environment for Statistical Computing*.
816 Vienna: R Foundation for Statistical Computing. Available online at: [https://www.R-](https://www.R-project.org/)
817 [project.org/](https://www.R-project.org/)
818
819 Rodríguez-Blanco, A., Giménez, M., 2019. Early effects of land use intensity on
820 arbuscular mycorrhizal fungi in rice-soybean rotations. In: D. Zúñiga-Dávila, F.
821 González-Andrés, E. Ormeño-Orrillo (eds.), *Microbial Probiotics for Agricultural*
822 *Systems. Sustainability in Plant and Crop Protection*, Springer Nature Switzerland.
823 https://doi.org/10.1007/978-3-030-17597-9_12
824
825 Selvakumar G., Panneerselvam P., Ganeshamurthy A., 2014. Diversity utility and
826 potential of Actinobacteria in the Agro-Ecosystem. In: Maheshwari D. (eds) *Bacterial*
827 *diversity in sustainable agriculture. Sustainable Development and Biodiversity*, vol 1.
828 Springer, Cham. https://doi.org/10.1007/978-3-319-05936-5_2
829
830 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B.,
831 Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B.,

832 Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source,
833 platform-independent, community-supported software for describing and comparing
834 microbial communities. *Applied and Environmental Microbiology* 75,7537–7541.
835

836 Schmidt, R., Mitchell, J. P., Scow, K., 2019. Cover cropping and no-till increase
837 diversity and symbiotroph:saprotroph ratios of soil fungal communities. *Soil Biology and*
838 *Biochemistry* 129, 99-109. <https://doi.org/10.1016/j.soilbio.2018.11.010>
839

840 Stewart, S.; Rodriguez, M. 2016. Manual de identificación de enfermedades de la soja
841 (2da. ed. ampliada y corregida). INIA Boletín de Divulgación N° 104.
842

843 Ter Braak, C.J.F., 1986. Canonical correspondence analysis: a new eigenvector
844 technique for multivariate direct gradient analysis. *Ecology* 67(5), 1167–1179.
845

846 Terra, J.A., Castillo, J., Saldain, N., Martinez, S., Bermúdez, R., Hernandez, J.,
847 Macedo, I., 2014. Rotaciones arroceras: resumen de resultados productivos en las
848 primeras zafras. In: INIA Treinta y Tres. Arroz-Soja: Resultados experimentales 2013-
849 2014. Treinta y Tres (UY: INIA Treinta y Tres. cap. 9. p. 22-24 (INIA Serie Actividades
850 de Difusión 735).
851

852 Turner, S., Pryer, K. M., Miao, V. P. W., Palmer, J. D., 1999. Investigating deep
853 phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA
854 sequence analysis. *The Journal of Eukaryotic Microbiology* 46, 327–338.
855

856 Venter, Z. S., Jacobs, K., Hawkins, H. J., 2016. The impact of crop rotation on soil
857 microbial diversity: A meta-analysis. *Pedobiologia* 59(4), 215-223.
858 <https://doi.org/10.1016/j.pedobi.2016.04.001>.
859

860 Wang, J., Chapman, S.J. & Yao, H., 2015. The effect of storage on microbial activity
861 and bacterial community structure of drained and flooded paddy soil. *Journal of Soils*
862 *and Sediments* 15, 880–889. <https://doi.org/10.1007/s11368-014-1053-7>.
863
864 Wright, A. F., Bailey, J.S., 2001. Organic carbon, total carbon, and total nitrogen
865 determinations in soils of variable calcium carbonate contents using a Leco CN-2000
866 dry combustion analyzer. *Communications in Soil Science and Plant Analysis*, 32, 3243
867 – 3258. <https://doi.org/10.1081/CSS-120001118>
868
869 Wu, M., Qin, H., Chen, Z., Wu, J., Wei, W., 2011. Effect of long-term fertilization on
870 bacterial composition in rice paddy soil. *Biology and Fertility of Soils* 47,397-405.
871 <https://doi.org/10.1007/s00374-010-0535-z>.
872
873 Wu, L., Ma, H., Zhao, Q., Zhang, S., Wei, W., Ding, X., 2020. Changes in soil bacterial
874 community and enzyme activity under five years straw returning in paddy soil.
875 *European Journal of Soil Biology* 100, 103215.
876 <https://doi.org/10.1016/j.ejsobi.2020.103215>.
877
878 Xuan, D. T., Guong, V. T., Rosling, A., Alström, S., Chai, B., Högberg, N., 2012.
879 Different crop rotation systems as drivers of change in soil bacterial community
880 structure and yield of rice, *Oryza sativa*. *Biology and Fertility of Soils* 48, 217–225.
881 <https://doi.org/10.1007/s00374-011-0618-5>.
882
883 Zhao, J., Ni, T., Xun, W., Huang, X., Huang, Q., Ran, W., Shen, B., Zhang, R., Shen,
884 Q., 2017. Influence of straw incorporation with and without straw decomposer on soil
885 bacterial community structure and function in a rice-wheat cropping system. *Applied*
886 *Microbiology and Biotechnology* 101, 4761–4773. [https://doi.org/10.1007/s00253-017-](https://doi.org/10.1007/s00253-017-8170-3)
887 8170-3.

888

889 Zheng, Q., Hua, Y., Zhang, S., Noll, L., Böckle, T., Dietrich, M., Herbold, C.W.,

890 Eichorst, S.A., Woebkenb, D., Richter, A., Waneka, W., 2019. Soil multifunctionality is

891 affected by the soil environment and by microbial community composition and diversity.

892 Soil Biology and Biochemistry 136, 07521.

893 <https://doi.org/10.1016/j.soilbio.2019.107521>.

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897 **Figure captions**

898

899 **Fig. 1.** Scheme of the phases of the study site sampled at the Long-time experiment of
900 Unidad Experimental de Paso de la Laguna, Treinta y Tres, Uruguay. Crop rotations,
901 names and codes, and crop per season and year before soil sampling (SS= spring-
902 summer, AW= autumn-winter, P= pasture).

903

904 **Fig.2.** Relative abundance found of a) bacterial phyla, and b) fungal classes according
905 with rotation studied. Rotations: CR=continuous rice, RC=rice and crops, RSP=rice and
906 short pasture, RP=rice and pasture (after pasture), RP2=rice and pasture (after rice),
907 RCP=rice, crops and pasture, and RS=rice and soybean (see Fig. 1).

908

909 **Fig. 3.** Relative abundance found of bacterial phyla *Proteobacteria* and *Verucomicrobia*
910 according with rotation studied. Rotations: CR=continuous rice, RC=rice and crops,
911 RSP=rice and short pasture, RP=rice and pasture (after pasture), RP2=rice and
912 pasture (after rice), RCP=rice, crops and pasture, and RS=rice and soybean (see Fig.
913 1). Bars represents standard errors based in three replications and different letters
914 above each bacterial phylum indicate significant differences ($P<0.05$) according with
915 Fisher's LSD.

916

917 **Fig. 4.** Relative abundance found of fungal classes *Cryptomycota*, *Glomeromycota* and
918 *Incertae sedis* according with rotation studied. Rotations: CR=continuous rice, RC=rice
919 and crops, RSP=rice and short pasture, RP=rice and pasture (after pasture), RP2=rice
920 and pasture (after rice), RCP=rice, crops and pasture, and RS=rice and soybean (see
921 Fig. 1). Bars represents standard errors based in three replications and different letters
922 above each fungal class indicate significant differences ($P<0.05$) according with
923 Fisher's LSD.

924

925

926 **Fig. 5.** Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis
927 distance for differences in composition of prokaryotic communities in the different
928 rotations studied (see Fig. 1). Rotations: CR=continuous rice, RC=rice and crops,
929 RSP=rice and short pasture, RP=rice and pasture (after pasture), RP2=rice and
930 pasture (after rice), RCP=rice, crops and pasture, and RS=rice and soybean (Stress:
931 0.196).

932

933 **Fig. 6.** Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis
934 distance for differences in composition of fungal communities in the different rotations
935 studied (see Fig. 1). Rotations: CR=continuous rice, RC=rice and crops, RSP=rice and
936 short pasture, RP=rice and pasture (after pasture), RP2=rice and pasture (after rice),
937 RCP=rice, crops and pasture, and RS=rice and soybean (Stress: 0.16).

938

939 **Fig. 7.** Canonical correspondence analysis (CCA) of bacterial OTUs in relation to
940 measured soil physico-chemical parameters.

941

942 **Fig. 8.** Canonical correspondence analysis (CCA) of fungal OTUs in relation to
943 measured soil physico-chemical parameters.

944

945 **Fig. 9.** Linear discriminant analysis Effect Size (LEfSe) (log10 LDA score) of
946 prokaryotic (Archaea/Bacteria) OTUs, which most likely explain differences between
947 rice rotations after a complete cycle of six years. Archaea/Bacteria OTUs are classified
948 at the highest resolvable taxonomic level. Rotations: CR= continuous rice, RC=rice and
949 crops, RSP=rice and short pasture, RP= rice and pasture (after pasture), RP2= rice
950 and pasture (after rice), RCP=rice, soybean and pasture, RS= rice and soybean.

951

	Season Years	1		2		3		4		5		6	
		2012-2013		2013-2014		2014-2015		2015-2016		2016-2017		2017-2018	
Name	Code	SS	AW	SS	AW	SS	AW	SS	AW	SS	AW	SS	AW
Continuous rice	CR	Rice	P	Rice	P	Rice	P	Rice	P	Rice	P	Rice	P
Rice and crops	RC	Rice	P	Sorghum	P	Rice	P	Soybean	P	Rice	P	Sorghum	P
Rice and short pasture	RSP	Rice	P	P	P	Rice	P	P	P	Rice	P	P	P
Rice pastures (rice 1)	RP1	P	P	Rice	P	Rice	P	P	P	P	P	P	P
Rice pastures (rice 2)	RP2	Rice	P	Rice	P	P	P	P	P	P	P	Rice	P
Rice, soybean and pasture	RCP	Rice	P	Soybean	P	Soybean	P	Rice	P	P	P	P	P
Rice and soybean	RS	Rice	P	Soybean	P	Rice	P	Soybean	P	Rice	P	Soybean	P

a Bacterial taxa

b Fungal taxa

Table 1. Soil chemical parameters evaluated for each of the rotations studied after six years at the Long-term experiment of Paso de la Laguna, Treinta y Tres, Uruguay. Rotations: CR= continuous rice, RC=rice and crops, RSP=rice and short pasture, RP= rice and pasture (after pasture), RP2= rice and pasture (after rice), RCP=rice, soybean and pasture, RS= rice and soybean. Values are means $\pm$ standard deviation of three replicates. Different letters indicate statistically significant differences between rotations ($P < 0.05$).

Rotation	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Organic Nitrogen (%)	Organic Carbon (%)	Organic Matter (%)	C:N	pH
CR	281.30 $\pm$ 59.27a	5.59 $\pm$ 1.69	28.40 $\pm$ 1.74	0.15 $\pm$ 0.01	1.31 $\pm$ 0.14	2.25 $\pm$ 0.24	8.68 $\pm$ 0.12a	5.86 $\pm$ 0.27
RC	850.37 $\pm$ 230.66c	6.24 $\pm$ 1.37	26.00 $\pm$ 1.06	0.19 $\pm$ 0.01	1.69 $\pm$ 0.09	2.91 $\pm$ 0.16	8.85 $\pm$ 0.17ab	5.43 $\pm$ 0.08
RSP	520.57 $\pm$ 79.27ab	6.76 $\pm$ 1.04	24.80 $\pm$ 1.06	0.21 $\pm$ 0.01	2.09 $\pm$ 0.15	3.60 $\pm$ 0.26	10.17 $\pm$ 0.47c	5.68 $\pm$ 0.05
RP	523.80 $\pm$ 78.40abc	6.63 $\pm$ 1.41	26.80 $\pm$ 0.80	0.17 $\pm$ 0.03	1.63 $\pm$ 0.33	2.80 $\pm$ 0.57	9.42 $\pm$ 0.36abc	5.68 $\pm$ 0.26
RP2	349.20 $\pm$ 73.45a	7.54 $\pm$ 2.03	27.20 $\pm$ 0.40	0.17 $\pm$ 0.01	1.69 $\pm$ 0.06	2.90 $\pm$ 0.10	9.78 $\pm$ 0.09bc	5.80 $\pm$ 0.09
RCP	355.67 $\pm$ 50.82a	8.32 $\pm$ 2.39	28.80 $\pm$ 1.83	0.20 $\pm$ 0.05	1.97 $\pm$ 0.58	3.39 $\pm$ 0.99	9.49 $\pm$ 0.60abc	6.03 $\pm$ 0.42
RS	753.37 $\pm$ 69.57bc	9.23 $\pm$ 0.13	30.00 $\pm$ 2.08	0.15 $\pm$ 0.01	1.33 $\pm$ 0.05	2.29 $\pm$ 0.09	8.66 $\pm$ 0.14a	5.69 $\pm$ 0.05
Significance	0.0162	ns	ns	ns	ns	ns	0.0415	ns

Table 2. Diversity indices at the OTU level of prokaryotic communities (archaea/bacteria) for each rotation studied. Rotations: CR = continuous rice, RC = rice and crops, RSP = rice and short pasture, RP = rice and pasture (after pasture), RP2 = rice and pasture (after rice), RCP = rice, soybean and pasture, RS = rice and soybean. Values are means $\pm$ standard deviation. Different letters indicate statistically significant differences between rotations ($P < 0.05$).

	CR	RC	RSP	RP	RP2	RCP	RS
Observed species	1814 $\pm$ 31a	1658 $\pm$ 28b	1780 $\pm$ 25ab	1683 $\pm$ 65ab	1726 $\pm$ 21ab	1753 $\pm$ 15ab	1704 $\pm$ 34ab
Shannon	5.839 $\pm$ 0.05	5.634 $\pm$ 0.04	5.699 $\pm$ 0.014	5.702 $\pm$ 0.03	5.683 $\pm$ 0.06	5.786 $\pm$ 0.04	5.690 $\pm$ 0.04
Simpson	0.991 $\pm$ 0.0001	0.989 $\pm$ 0.0001	0.989 $\pm$ 0.001	0.990 $\pm$ 0.001	0.990 $\pm$ 0.001	0.991 $\pm$ 0.001	0.989 $\pm$ 0.001
Chao1	2189 $\pm$ 18	2073 $\pm$ 9	2188 $\pm$ 7	2115 $\pm$ 66	2115 $\pm$ 9	2118 $\pm$ 34	2153 $\pm$ 44
ACE	2218 $\pm$ 37	2115 $\pm$ 20	2246 $\pm$ 13	2142 $\pm$ 64	2168 $\pm$ 23	2162 $\pm$ 29	2147 $\pm$ 38

Table 3. Diversity indices at the OTU level of fungal communities for each rotation studied. Rotations: CR = continuous rice, RC = rice and crops, RSP = rice and short pasture, RP = rice and pasture (after pasture), RP2 = rice and pasture (after rice), RCP = rice, soybean and pasture, RS = rice and soybean. Values are means $\pm$ standard deviation. Different letters indicate statistically significant differences between rotations ($P < 0.05$).

	CR	RC	RSP	RP	RP2	RCP	RS
Observed_species	292 $\pm$ 12	322 $\pm$ 30	329 $\pm$ 9	321 $\pm$ 15	343 $\pm$ 13	298 $\pm$ 36	299 $\pm$ 21
Shannon	2.69 $\pm$ 0.17b	2.99 $\pm$ 0.08a	2.98 $\pm$ 0.05a	2.63 $\pm$ 0.10b	3.03 $\pm$ 0.10a	2.83 $\pm$ 0.08ab	3.06 $\pm$ 0.04a
Simpson	0.826 $\pm$ 0.049	0.891 $\pm$ 0.012	0.896 $\pm$ 0.002	0.836 $\pm$ 0.010	0.900 $\pm$ 0.013	0.875 $\pm$ 0.013	0.893 $\pm$ 0.007
Chao1	401 $\pm$ 22	441 $\pm$ 26	427 $\pm$ 8	423 $\pm$ 25	426 $\pm$ 9	399 $\pm$ 38	399 $\pm$ 8
ACE	407 $\pm$ 31	417 $\pm$ 45	432 $\pm$ 11	426 $\pm$ 16	426 $\pm$ 12	419 $\pm$ 24	378 $\pm$ 18