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Impacts of protected colonial birds on soil microbial communities: When protection leads to degradation — Source link

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2 **communities: when protection leads to degradation**

3

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13

14 **Abstract**

15 Colonial nesting and roosting birds can degrade their habitat by soil salinization,
16 eutrophication, and acidification associated with excessive deposition of avian excreta.
17 We studied the impact of a protected wading bird colony on soil microbial communities
18 from cork oak woodlands in Doñana National Park (SW Spain). Over one year we
19 analyzed soil properties (pH, salinity, soluble N and P forms, extractable organic carbon
20 - EOC -), microbial activity (basal respiration, community-level physiological profile,
21 extracellular enzyme activities) and community structure (fungal, bacterial and archaeal
22 terminal restriction fragments -TRFs-) along a gradient of bird nesting intensity. Bird

nesting largely impacted soil chemical environment, with increases from 25 to 500 μS cm^{-1} in soil salinity, from 6 to 725 mg kg^{-1} in soil P, from 5 to 22 mg kg^{-1} in N-NH_4, and from 5.4 to 245 mg kg^{-1} in N-NO_3 between the extremes of the nesting intensity gradient in the wet season. Most of these chemical changes were enhanced in the dry season. We observed positive linear or log-linear relationships between the bird nesting footprint on soils (indicated by an integrated soil chemistry index) and microbial biomass, basal respiration and most of the studied enzyme activities. This was likely due to the concurrent increases in EOC along the avian intensity gradient, which counteracted the negative impacts of salinity. Soil P and EOC were the main drivers for fungal, bacterial and archaeal TRFs diversity. Bacterial TRFs richness and diversity index decreased along the avian intensity gradient in the dry season, while archaeal TRFs diversity increased in those soils highly salinized by excess of avian excreta deposition. Our study clearly shows that this oversized bird colony has profound effects on soil chemistry and biological activity, and highlights the need for a re-evaluation of management strategies in the area, towards a greater consideration of soil processes in conservation priorities.

Key words: guano, soil enzymes, soil respiration, cork oak, TRFLP

Highlights

- The impact of a protected bird colony on soil microbial communities was studied
- Birds largely impacted soil chemistry and microbial community structure and function
- Bacterial diversity decreased with avian intensity, and archaeal diversity increased

47 • Nesting birds compromised the maintenance of the native soil microbial
48 communities

49

50 ## 1. Introduction

51 Oversized animal populations may have profound effects on soil biogeochemical cycles
52 in terrestrial ecosystems, for instance by reducing C inputs belowground due
53 overgrazing (Raiesi and Asadi, 2006; Mchunu and Chaplot 2012), and by providing
54 nutrient inputs through faecal deposition. This is the case of those systems used by birds
55 for nesting or roosting, where soil acidification, eutrophication and salinization might
56 occur due to the deposition of large amounts of guano, which is highly enriched in N
57 and P salts (García et al., 2002a, 2002b; Ligeza and Smal, 2003; Zwolicki et al., 2013).
58 The increase in waterbird protection initiatives and the drastic expansion of seagulls
59 during the last two decades have resulted in a growing number of areas affected by
60 ornithogenic soil degradation, which has been reported in Mediterranean and Atlantic
61 islands (García et al., 2002b; Baumberger et al., 2012; Otero et al., 2015), forest and
62 wetland ecosystems in Europe (Zólkoś et al., 2006; Kutorga et al., 2013), the Great
63 Lakes in North America (Hebert et al., 2005) or lakeside forests and islands in Australia
64 (Baxter and Fairweather, 1994; Bancroft et al., 2005) and Japan (Hobara et al., 2005;
65 Katsumata et al., 2015).

66 Piscivorous birds transport N and P from their wide feeding areas into their nesting and
67 roosting habitats, which might release plant and soil microbial nutrient deficiencies in
68 environments with nutrient-poor soils (Speir and Ross, 1984; Wait et al., 2005;
69 Tscherko et al., 2003; Sigurdsson and Magnusson, 2010; Korsten et al., 2013; Adame et
70 al., 2015; Irick et al., 2015). However, in those systems where the size of the bird

71 colony is too large, detrimental effects on plant or soil microbial communities may
72 occur due to the excess of nutrients and salts in soils (García et al., 2002a; Ellis, 2005;
73 Ellis et al., 2006; Wait et al., 2005). Excess of guano deposition can lead to N saturation
74 in soils, resulting in C limitations for soil microbes that enhances organic matter
75 decomposition (Hawke et al., 2015), or in decreased rates of litter decomposition by
76 fungi due to the formation of acid-insoluble lignin-like substances complexes in plant
77 biomass to immobilized excreta-derived N (Osono et al., 2006a).

78 Some studies have shown that excessive avian excreta inputs can alter the structure of
79 soil fungal and bacterial communities, with decreases in fungal growth (Osono et al.,
80 2002, 2006b), mycorrhizal and myxomycetous richness (Adamonytė et al., 2013;
81 Kutorga et al., 2013), and the fungi:bacteria PLFA ratio (Wright et al., 2010) in those
82 soils highly fertilized by birds, in comparison to nearby control sites. Most of these
83 studies, however, have focused mainly on a single microbial group, and information
84 about the relative impact of guano deposition on the diversity of different microbial
85 groups is still lacking. While bacteria might be more affected than fungi by changes in
86 soil pH (Lauber et al., 2008; Rousk et al., 2010), fungi appear to be more affected by
87 increased salinity and nutrient inputs than bacteria, as suggested by several studies
88 reporting decreases in fungal dominance in response to salinization (reviewed in Rath
89 and Rousk, 2015) and long-term N or P fertilization (Bradley et al., 2006; Wallestein et
90 al., 2006; Demoling et al., 2008; Rousk et al., 2011a). The impact of fertilization by
91 birds on archaeal community remains to be studied. Recent works have reported a
92 greater sensitivity of archaeal than bacterial and fungal communities to disturbances in
93 soils with a long history of N-fertilization (Pereira e Silva et al., 2012), so it is likely
94 that the archaeal community is highly responsive to fertilization of the soils from these
95 bird habitats.

96 Here, we analyzed soil microbial activity and diversity along a gradient of guano
97 deposition in a large colony of wading birds established in a cork oak woodland at the
98 Doñana National Park (SW Spain). This park is one of the most important bird reserves
99 in Europe, where a large colony of wading birds established on the cork oaks and other
100 tree species located in the ecotone between the woodland and the marshland in the
101 1947-1948 (Bernis and Valverde 1954). Since then, the increase in the size of the bird
102 colony has resulted in some detrimental effects on the trees. In a previous study we
103 showed that the observed cork oak decay in this woodland was explained by the effects
104 of nesting birds on soils (mainly soil salinization and fertilization, García et al, 2011).
105 After 46 years of bird protection, the risk of death to centenarian and planted cork oaks
106 in the area occupied by the bird colony was over twofold higher than for trees outside
107 the nesting area (Fedriani et al., 2016).

108 We explored the ornithogenic impact on soil microbial communities during two
109 contrasting seasons, through the analysis of their activity (basal respiration,
110 mineralization of low molecular weight C compounds and enzyme activities), and
111 diversity (fingerprint profiling of fungi, bacteria and archaea) along a gradient of bird
112 nesting intensity. We expected that in these naturally acidic and N- and P-poor soils,
113 where microbial biomass and litter decomposition are strongly limited by a low P
114 availability (Aponte et al., 2010, 2012), moderate inputs of guano would increase N
115 and P availability, resulting in increased microbial biomass, basal respiration and
116 extracellular enzyme activities. Under high nesting intensity conditions, however, we
117 expected that the excess of salts and nutrients would negatively impact these variables.
118 Thus, our first hypothesis is that the positive relationships between bird input intensity
119 and microbial activity variables are not linear, but peak at intermediate levels of bird
120 input intensity. Likewise, we expected a higher richness of Terminal Restriction

121 Fragments (TRFs) at intermediate nesting intensities, but a decrease in richness at high
122 bird nesting intensities due to the strong selective pressure imposed by soil acidification,
123 hypersalinization and hyperfertilization. We expected that the changes would be
124 particularly marked for the fungal community, in agreement with the reported decreases
125 in fungal dominance in soils in response to fertilization and salinization (Wallestein et
126 al., 2006; de Vries et al., 2006; Rath and Rousk, 2015). Thus, our second hypothesis is
127 that the decline in TRF diversity under high nesting intensity conditions is stronger for
128 fungi than for bacteria or archaea.

129

130 **2. Material and Methods**

131 2.1. Study site

132 Doñana National Park is located in SW Spain, and comprises about 30,000 ha of clayey
133 marshlands and about 25,000 ha of dunes, sparse forests and shrublands on sandy soils
134 (Montes et al., 1998). This National Park is one of the main wintering areas for birds in
135 Europe (Rendón et al. 2008), as well as one of the most important areas for waterbird
136 nesting in Western Europe (Ramo et al. 2013). The park is protected since 1969 and was
137 declared as Biosphere Reserve in 1981 and as World Heritage Site in 1994. Climate is
138 Mediterranean with an average annual rainfall of 550 mm and an average temperature
139 of 16–17 º C.

140 Cork oaks in the area grow on acidic, nutrient-poor sandy soils, and are formed by a few
141 thousand scattered centenarian trees (savannah-like woodland). Our study was
142 conducted in the ecotone between the woodland and the marshland, which is locally
143 known as “La Vera de Doñana”, where a colony of wading birds established in 1947-
144 1948. The colony, ranging from 150 to 13000 pairs of birds depending on the marsh

145 flood level (Ramo et al., 2013), is composed by seven species: white stork (*Ciconia*
146 *ciconia*), spoonbill (*Platalea leucorodia*), grey heron (*Ardea cinerea*), little egret
147 (*Egretta garzetta*), cattle egret (*Bubulcus ibis*), squacco heron (*Ardeola ralloides*) and
148 black-crowned night-heron (*Nycticorax nycticorax*). Birds intensively use cork oak trees
149 as nesting sites, which often results in physical damage to the trees as well as in
150 increases in soil salinity due to guano deposition (García et al., 2011).

151 2.2. Sampling design

152 Soil sampling was conducted in autumn 2012 and repeated at end of summer 2013,
153 outside the nesting season (February to July). During the autumn (wet season), the
154 influence of the bird colony on soil processes was expected to be lower, due to the
155 leaching of guano during rainy events. Maximum accumulation of guano was expected
156 to occur by the end of summer, just after the end of the nesting season and coinciding
157 with the period of lowest rainfall.

158 The study was conducted across three levels of bird nesting intensity (low, medium,
159 high), established based on the records of tree occupation by birds during the 1998-2012
160 period. Trees in the high nesting intensity (HNI) category had been used by birds during
161 at least the last 13-15 years, with a number of nests per tree per year ranging between 12
162 and 75; trees under medium nesting intensity (MNI) had been occupied for 7-12 years,
163 with an average annual occupation per tree ranging from 6 to 26 nests; trees in the low
164 nesting intensity category (LNI) had had none to less than 2 nests per tree annually,
165 been occupied by birds for a maximum of 4 years between 1998 and 2012.

166 In each category five cork oak trees were selected, and soils were sampled underneath
167 the tree cover in three different positions (N, SE and SW) at a 2 m distance from the
168 trunks. In each of these positions, a 1 m^2 quadrat was established, and three soil samples

169 (0-10 cm depth) were collected along the diagonal line of the quadrat using a core of 5
170 cm of diameter. A composite sample for each quadrat was then obtained by mixing
171 these three soil samples. Soil samples from each of the three positions sampled
172 underneath each tree were analyzed separately, to account for the intra-site
173 heterogeneity. The total number of soil samples was 45 for each sampling period.

174 2.3. Soil processing and chemical analysis

175 Soils were transported to the lab into a refrigerated container. Immediately after
176 returning from the field a subsample of each soil was sieved to < 2 mm and frozen to -
177 80 ºC for T-RFLP (Terminal-Restriction Fragment Length Polymorphism) analysis. A
178 second subsample was sieved to < 2 mm and kept in the dark at 4 ºC until analysis of
179 microbial biomass, enzyme activities and community-level physiological profile. The
180 rest of the sample was air-dried, sieved to < 2 mm and used for chemical analysis.

181 Soil pH and salinity (electric conductivity) were measured in 1:5 (soil: water) extracts.
182 Organic matter content was determined by the loss of ignition method, after burning the
183 sample at 540 ºC for 3 h. Nitrate and ammonium content in 1M KCl soil extracts were
184 determined spectrophotometrically. Phosphorous was analyzed using the Bray and
185 Kurtz method (Bray and Kurtz, 1945). Organic carbon content in 0.5M K_2SO_4 soil
186 extracts (Extractable Organic C -EOC-) was determined using a Shimadzu TOC-V SCH
187 analyzer.

188 2.5. Soil microbial biomass and activity

189 Soil microbial biomass C and N was estimated using the chloroform fumigation-
190 extraction method (Vance et al., 1987). The potential activity of several extracellular
191 enzymes (EEAs) involved in C, S and P cycling (β-glucosidase, aryl-sulphatase and

192 acid phosphatase) was analyzed colorimetrically by incubation of 1 g of soil with p-
193 nitrophenyl-linked substrates at 37 ºC during 1 hour following the procedures by Eivasi
194 and Tabatabai et al. (1988) and Tabatabai and Bremmer (1969, 1970). Urease activity
195 was determined as the rate of ammonium release after incubation of 1 g of soil with 80
196 mM urea at 37 ºC during 2 hours (Kalender et al., 1999). Mass-specific enzyme
197 activities were calculated as the ratio between enzyme activities and microbial C.

198 In the set of soil samples collected in the wet season, the community-level physiological
199 profile (CLPP) was analyzed using the MicroResp system (Campbell et al., 2003).
200 Fifteen C-compounds belonging to different chemical groups (sugars, amino acids,
201 amines and carboxylic acids) were added to soils in 96-well microtiter deep well plates
202 (final volume of 400 μL of soil per well), at a concentration of 30 mg of substrate per
203 ml of soil water. Previously, soil water content was determined gravimetrically, and the
204 amount of substrate to be added was calculated to have final water contents close to 60
205 % of soil water holding capacity during the assay. The respiration induced by the added
206 substrates was determined after 6 h of incubation at 25 ºC by absorbance measurements
207 in detector plates, consisting of 96-well microplates filled with cresol red agar: a
208 mixture of an indicator solution (18.75 ppm cresol red dye, 220 mM potassium chloride
209 and 3.75 mM sodium bicarbonate) amended with melted 3% purified agar (2:1
210 indicator:agar). All soils were pre-incubated at 25 ºC during 24 h before running the
211 assays. Basal respiration was measured simultaneously, in wells filled with soils at the
212 same water content, but without substrate addition. Metabolic quotient (qCO_2) was
213 calculated as the ratio between basal respiration and microbial C. For each sample
214 Shannon index ($H = -\sum p_i \ln p_i$) was computed from substrate-induced respiration rates,
215 as a measure of functional diversity, where p_i is the proportional respiration induced by
216 a particular i substrate.

217 2.6. T-RFLP analysis of soil fungi, bacteria and archaea

218 Total DNA was extracted from 0.5 g of soil using a *i-genomic soil DNA Extraction Mini*
219 *Kit* (iNtRON Biotechnology, Korea). The DNA was quantified fluorometrically in a
220 microplate reader (FLUOstar Omega, BMG Labtech, Germany) using *Quant-iT*™
221 *PicoGreen*® reagent (Invitrogen, Carlsbad, CA, EEUU). The extracted DNA was then
222 amplified by a Polymerase Chain Reaction procedure (PCR). Bacteria, archaea and
223 fungi were amplified separately, using two primers for each microbial group combined
224 in the same reaction. Bacterial (16S rRNA), archaeal (16S rRNA) and fungal (ITS)
225 sequences were amplified according to Sign et al. (2006). Each PCR reaction consisted
226 of a 20 µl reaction mixture containing 1 µl of DNA, 4 µl of 5x *MyTaq Red* buffer
227 (Bioline GmbH, Luckenwalde, Germany), 0.25 µl of *MyTaq* DNA polymerase (final
228 concentration 1.25 U, Bioline GmbH, Luckenwalde, Germany), 0.08 µl of 10% Bovine
229 Serum Albumin (Sigma, Poole, UK) and aliquots of pairs of primers for either bacteria
230 (63f and 1087r-VIC labelled, at a final concentration of 50 nM; Marchesi et al., 1998;
231 Hauben et al. 1997), archaea (Ar3f and AR927r-PET labelled, at a final concentration of
232 800 nM; Giovannoni et al. 1988; Jurgens et al. 1997) or fungi (ITS1f-FAM labelled and
233 ITS4f at a final concentration of 400 nM; Gardes and Bruns 1993; White et al., 1990),
234 all primers sourced from Life Technologies (Carlsbad, CA, EEUU). Reactions were
235 conducted in a MultiGENE Optimax thermocycler, with the following protocol: initial
236 step of 2 min at 94 ºC, 40 cycles of denaturating of 10 s at 94 ºC, annealing for 30 s at
237 54º C, elongation for 1 min at 72 ºC, and a final extension period of 5 min at 72 ºC.
238 Amplification was verified visually using electrophoresis on a 1.3% agarose gel with a
239 RedSafe™ nucleic acid staining solution (iNtRON Biotechnology, Sungnam, Corea).
240 Amplified PCR products were purified using the FavorPrep™ PCR Clean-Up Kit

241 (Favorgen Biotech Corp, Ping-Tung, Taiwan) and quantified using Quant-iT™
242 PicoGreen®.
243 Cleaned PCR products (1500 ng) were digested in a 40 µl reaction mixture consisting of
244 4 µl of 10 x Tango buffer and a aliquot of HhaI restriction enzyme to a final
245 concentration of 5 U (reagents sourced from Fermentas UAB, Vilnius, Lithuania),
246 during 3 h at 37ºC. Products were purified using the MinElute PCR Purification Kit
247 (Qiagen, Hilden, Germany), and quantified again using Quant-iT™ PicoGreen®. After
248 purification, aliquots of each sample were sent to Secugen SL (Madrid, Spain) for
249 analysis of Terminal Restriction fragments (TRFs) on an ABI3730 Genetic Analyzer
250 (Applied Biosystems, Foster City, CA, USA). The size of the TRFs was determined
251 using GeneScan™ 500 LIZ® Size Standard (Applied Biosystems, Foster City, CA,
252 USA). TRFs between 50 and 500 base pair length with amplitude greater than 50
253 fluorescent units were analyzed using Peak Scanner v1.0 (Applied Biosystems, Foster
254 City, CA, USA). Relative abundances were calculated from peak fluorescence values.
255 TRFs data was finally examined and filtered using T-REX software (Culman et al.,
256 2009). For each sample Shannon diversity index was computed from fungal, bacterial
257 and archaeal TRFs, as described above.
258
259 2.7. Data analysis
260 Data analysis was conducted using different packages in R 3.1.2. Linear or generalized
261 linear mixed models were applied after exploration of frequency distributions of soil
262 chemical variables to test for differences among nesting intensity categories, using the
263 nlme (mixed linear models, LMM) or the lme4 (generalized linear mixed models,
264 GLMM) packages. Nesting intensity was set as fixed factor, and tree identity as random

265 factor. Tukey post-hoc tests were applied to confirm differences among nesting
266 intensity categories.

267 Heterogeneity in soil chemical properties within bird nesting categories and among
268 samples collected underneath individual trees was explored by calculating coefficients
269 of variation. The high heterogeneity of soil salinity and nutrients within the MNI and
270 HNI categories suggested a large variability in the impact of our a-priori variable
271 (historical nesting intensity recorded at each sample tree) on soils. In order to explore
272 changes in soil microbial activity and community structure along a continuous gradient
273 of bird influence, rather than among nesting intensity categories, we conducted a
274 Principal Component Analysis (PCA) of soil abiotic variables (individual samples) for
275 each season, to extract an integrated continuous index of avian influence for each
276 datasets. For both datasets the first PCA component integrated the main soil chemical
277 gradient (Table 1), and was used thereafter as a Bird Chemical Footprint (BCF) index.
278 To test our hypotheses about the impact of the bird influence on soil microbial
279 communities we also used this quantitative index, and explored the shape of the
280 relationships between microbial variables (microbial biomass, enzyme activities, basal
281 respiration, functional diversity and richness and diversity of fungal, bacterial and
282 archaeal TRFs) and a reduced set of soil chemical predictors (the integrated BCF and
283 soil pH) through a modelling approach, applying LMM, GLMM or additive mixed
284 models (see details of the modelling approach in Supplementary Information).

285 Multivariate analysis of CLPP and TRF profiles was performed with the vegan package
286 (Oksanen, 2015). Permutational ANOVA was applied to CLPP data, to test for the
287 influence of tree identity, BCF and soil pH, using the adonis function. Bacterial, fungal
288 and archaeal TRF datasets were analyzed separately for each sampling season. Hellinger
289 transformation was applied to each data set. First, unconstrained ordinations of the

290 abundance matrices were performed using Non-metric Multidimensional Scaling
291 (NMDS).The rankindex function in the vegan package was previously applied to each
292 data set to evaluate which of the distance indices best separated communities along the
293 environmental gradient considered (bird influence intensity), using rank correlations.
294 Euclidean distance was used for all data sets, except for bacterial and archaeal
295 communities in the dry season, for which Bray-Curtis and Manhattan distances were
296 used, respectively. Environmental vectors representing the studied soil abiotic variables
297 were fitted onto the ordination planes using the envfit function. Then, constrained
298 analysis (Redundancy Analysis, RDA) was applied to study the variation in TRFs
299 composition due to the main soil chemical gradient (BCF) and soil pH, with the block
300 factor (tree identity) introduced as conditional factor. We further conducted the RDA
301 analyses with a subset of individual soil chemical variables, instead of the integrated
302 index: pH, CE, N-NH_4, P, and organic matter (wet season) or extractable organic C (dry
303 season). The best combination of explaining variables was selected by applying a
304 forward model building procedure. Variance inflation factors were calculated for the
305 final models, to check for independency of the selected predictors.

306

307 **3. Results**

308 3. 1. Soil chemistry along the nesting intensity gradient

309 Nesting intensity did not have a significant influence on soil pH (Fig. 1a). Electrical
310 conductivity (EC), however, was highly influenced by bird nesting, being average EC
311 values 8 and 20 times higher in the MNI and HNI categories, respectively, in
312 comparison to the LNI category during the wet season (Table 1). Salinity increased
313 across the three nesting categories in the dry season, differences among categories being

314 reduced but still showing significant increases in the MNI and HNI categories in
315 relation to LNI (3 and 13 times higher, respectively).

316 These changes in soil salinity were related to exponential increases in the contents of N-
317 NH_4, N-NO_3 and P in soils (Table 1). In the wet season N-NH_4 content in the soils of the
318 HNI category was significantly higher than in those soils of the LNI category (on
319 average, 3.3 and 4.5 times higher, respectively. In the dry season soil N-NH_4 strongly
320 increased in comparison to autumnal levels, and differences among categories were
321 more pronounced, with maximums of 570 and 1570 mg N-NH_4 kg^{-1} for the MNI and the
322 HNI categories, respectively. Nitrate followed an opposite pattern, soil concentrations in
323 the wet season being greater that those in the dry season. In the wet season, N-NO_3
324 concentrations were up to 29 and 45 times greater in the MNI and HNI categories,
325 respectively, in comparison to LNI. Phosphorus showed the greatest changes between
326 the LNI and the MNI/HNI categories, which persisted over both the wet and the dry
327 season. Phosphorus concentrations were, on average, more than 60 and 115 times
328 greater in the MNI and the HNI categories than in the LNI category, respectively, at
329 both seasons.

330 Nesting intensity had also some effect on soil organic matter content in the wet season,
331 being significantly greater underneath trees exposed to a high nesting intensity in
332 comparison to the LNI category (Table 1). The increases in soil organic matter were
333 more pronounced in the dry season, just after the end of the nesting period. Likewise,
334 nesting intensity had a positive effect on Extractable Organic Carbon (EOC), soils
335 underneath trees exposed to a low nesting influence showing significantly lower
336 concentrations at both sampling times, in comparison to the HNI category (Table 1).
337 Relative increases in EOC were, however, much lower than those in N forms and P. In

338 the HNI category, average EOC concentrations were 3.5 times greater than in the LNI
339 category in the wet season, and 1.6 times greater in the dry season.

340 In the MNI and HNI categories the increases in soil electrical conductivity and nutrient
341 concentrations (mainly N-NH_4 and N-NO_3) were not homogeneous within the area
342 covered by individual trees. In contrast, coefficients of variation of these variables at the
343 tree level were > 60 % indicating the patchiness of guano accumulation onto soils (data
344 not shown). Multivariate analysis of soil abiotic variables applied to individual soil
345 samples showed that most of these chemical variables were mutually correlated. For
346 example, a strong link between EC and EOC was observed when individual samples
347 were pooled, particularly for the wet season (Fig. 1), suggesting that guano deposition
348 did not only supplied N and P to soils, but also labile C compounds. The first PCA
349 factor explained > 70 % of chemical soil variability, and included those soils variables
350 that more clearly responded to the bird nesting influence, namely EC, N and P salts,
351 EOC and OM, while pH followed an independent pattern of variation (Table 2).

352 3.2. Soil microbial biomass and activity response to guano deposition

353 Soil microbial biomass and basal respiration in these soils were clearly stimulated with
354 the avian intensity. The mixed models applied to microbial C and N, with the avian
355 chemical footprint index (BCF) and soil pH as predictors, showed that both variables
356 increased simultaneously along the avian intensity gradient, and that the C:N ratio
357 remained unaffected (Fig. 2; Supplementary Information Table S1). In the dry season
358 the response to the avian influence was greatest at soil pH < 6 (Fig. 2b, d).

359 Soil basal respiration ranged from 5 to 19 μg C-CO_2 g^{-1} h^{-1} between the extremes of the
360 guano deposition gradient. The response of soil basal respiration to BCF also depended
361 on soil pH, being more stimulated with increasing pH conditions (Fig. 3). In contrast,

362 the metabolic quotient (ratio of basal respiration to microbial biomass) was unaffected
363 by the avian influence (Supplementary Material, Table S1). For each of the 15 tested
364 substrates induced respiration also responded positively to guano deposition (correlation
365 coefficients between BCF and respiration rates ≥ 0.75, $p < 0.001$). The permutational
366 ANOVA applied to the CLPP data showed a significant effect of guano deposition (F=
367 440.7; $p = 0.001$), which explain 20 % of total inertia. However, functional diversity
368 (Shannon H index) remained unchanged (Supplementary Material, Table S1), meaning
369 that guano deposition enhanced the respiration induced by the tested C substrates
370 without changing the relative use of these substrates by soil microbes.

371 Enzyme activities were, in general, also stimulated by guano deposition, in particular
372 acid phosphatase, urease and β-glucosidase (Fig. 4). The models selected for aryl-
373 sulphatase activity also included BCF as a significant predictor, and showed a positive
374 but weaker response to BCF, in comparison to the rest of enzymes. Some enzyme
375 activities were also significantly influenced by soil pH, with a significant BCF × pH
376 interaction for urease during the wet season (Fig. 4e). The proportion of variance in
377 enzyme activity explained by pH and BCF ranged from 12 % to 75 % in the wet season,
378 and from 13 % to 63 % in the dry season (Supplementary Material, Table S1). Urease
379 was particularly responsive to BCF in the summer, showing a non-linear response
380 across the studied gradient (Fig. 4f). For this enzyme, seasonal differences were
381 especially high at the high end of the avian intensity gradient, summer urease activities
382 being up to 50 times greater than maximum autumnal activities. In contrast, the effect of
383 avian intensity on mass-specific enzyme activities was either null (urease) or negative
384 (acid phosphatase, aryl-sulphatase and β-glucosidase, Supplementary Material, Table
385 S1).

386 3.3. Microbial community composition

387 The changes in abiotic soil properties induced by the avian intensity shaped the
388 structure of the soil microbial communities. In general, the response to avian intensity
389 was clearer for archaea than for fungi or bacteria.

390 Fungal TRF richness and diversity (Shannon H index) did not change across the three
391 nesting intensity categories (Fig. 5a). However, the ordination resulting from the Non-
392 Metric Multidimensional scaling (NMDS) revealed a clear separation of the fungal
393 community between the LNI and the M/HNI categories, particularly in the wet season
394 (Fig. 6a). Indirect gradient analysis suggested that P availability was the soil abiotic
395 variable with the greatest influence on fungal community ordination. In the constrained
396 analysis (RDA) the integrated bird intensity index only explained a limited, non-
397 significant fraction of the variability in fungal TRFs (< 4 %, data not shown). However,
398 when a subset of soil chemical parameters were used as explaining variables, instead of
399 the integrated BCF, the selected model (forward model selection procedure) included
400 phosphorus availability as a significant explaining variable for the fungal community in
401 the wet season, which explained a 8% of total inertia (Table 3). In the dry season the
402 selected model included soil EOC, pH and P and accounted for a 14% of total inertia
403 (Table 3).

404 Bacterial community in the HNI category spanned broadly and overlapped over the
405 NMDS ordination plane with that in the MNI category in the wet season, but was
406 clearly separated from the community in the LNI category (Fig. 6c). Avian intensity
407 was a significant predictor of the bacterial community structure in this season; when
408 individual chemical variables were used as explaining variables, the selected model
409 included soil P and organic matter, which account for a 14.5 % of total inertia (Table 3).
410 In the dry season bacterial community in the HNI category was more clearly
411 differentiated from the community in the MNI category (Fig 6c). Bacterial TRF richness

412 and diversity decreased with increasing levels of BCF (Supplementatry Material, Table
413 S1). Redundancy analysis confirmed that at this season the structure of the bacterial
414 community was determined by some of those chemical parameters related to the avian
415 intensity, in particular by the amount of EOC (Table 3).

416 Likewise, archaeal community was also influenced by avian intensity at both seasons.
417 However, in contrast to bacteria, archaeal TRF diversity increased along the intensity
418 gradient (Fig. 8c, Supplementary Material Table S1), particularly in the wet season
419 when it was positively related to soil P (R^2 = 0.52, p = 0.0056). The NMDS clearly
420 differentiated between the LNI and the HNI categories at both seasons (Fig. 9e, f). The
421 most influential abiotic variables for the archaeal community were soil P (wet season)
422 and EOC (dry season), which explained 8.6 % and 7.6 % of the variability in archaeal
423 TRFs, respectively (Table 3).

424

425 **4. Discussion**

426 4.1 Impact of avian intensity on soil chemistry, microbial biomass and activity

427 Cork oak forests from SW Spain are characterized by nutrient-poor soils, where
428 microbial biomass and litter decomposition are strongly limited by a low P availability
429 (Aponte et al., 2010, 2012). Thus, we hypothesized that guano deposition, which is
430 highly enriched in N and P, would play a central role in the activity and structure of soil
431 microbial communities in the study cork oak woodland. We expected a stimulation of
432 soil microbial biomass, respiration and enzyme activities at intermediate levels of avian
433 intensity, and detrimental effects on these microbial variables at high levels of avian
434 intensity, due to the known adverse effects of salinization on soil microbial activity and

435 in agreement with the observations of tree health impairment in those sites highly used
436 by birds for nesting (García et al., 2011).

437 Indeed, in our study we detected increases of >10 times in soil $EC_{1:5}$, > 100 times in soil
438 P and 3-45 times (depending on the season) in $N\text{-}NH_4$ and $N\text{-}NO_3$ between the extremes
439 of the avian intensity gradient. At the dry season we recorded soil $EC_{1:5}$ values as high
440 as 4000 μS cm^{-1}. Similar increases in soil salinity have been reported to inhibit
441 microbial growth (Rousk et al., 2011b), decrease microbial biomass (Sardinha et al.,
442 2003; Tripahi et al., 2006), provoke a decline in soil respiration (Setia et al., 2011; Rath
443 and Rousk, 2015,) and impair extracellular enzyme production (García and Hernández
444 1996; Saviozzi et al., 2011) in a range on studies with different soil types. However, and
445 in contrast to our hypothesis, we observed positive linear or log-linear relationships
446 between the bird nesting footprint on soils (indicated by the integrated avian intensity
447 index) and microbial biomass, basal respiration and most of the studied enzyme
448 activities. Interestingly, the magnitude of the stimulation of microbial C and N and by
449 bird inputs was dependent on soil pH, remaining greater at pH values around 5.6 than at
450 pH above 6.3 (seasonal median) during the dry season. This suggests that pH conditions
451 close to 5.5 are optimal for microbial growth in these naturally-acidic forest soils.
452

453 The fact that microbial biomass and activity was not impaired at the high end of the
454 avian intensity gradient is likely due to the concurrent increases in extractable organic C
455 along this gradient (Fig. 4). It is well known that the addition of organic C to saline soils
456 minimizes the toxicity induced by salinization and increases microbial biomass and
457 respiration (Liang et al., 2005; Tejada et al., 2006; Wong et al. 2009). This positive
458 response of the microbial community can be very quick if the added substrate is labile C
459 (Wong et al., 2009). The increases in EOC concentrations along the studied gradient

460 could be due not only to the deposition of bird detritus, but also to the higher amount of
461 litterfall accumulated underneath the trees occupied by birds, since the intensive use of
462 these trees enhances tree defoliation (García et al., 2011).

463 Analysis of the community level physiological profile revealed increasing rates of
464 substrate-induced respiration along the guano deposition gradient. As found in others P-
465 limited forest ecosystems, P fertilization leads to an increase in the catabolic use of a
466 broad range of low-molecular weight C compounds (Fanin et al., 2015). In contrast,
467 functional diversity was stable, without changes in the relative use of the tested
468 substrates (sugars, amino acids, amines and carboxylic acids). Given that the increases
469 in labile N forms were much greater than the increases in labile organic C in those soils
470 under a high avian intensity, we could have expected a greater stimulation in the use of
471 C compounds than of aminoacids and amines in these soils, as found in some forest
472 soils in response to N fertilization (Lagomarsino et al., 2007). Probably, with a broader
473 range of substrates, including also more complex, recalcitrant C compounds, we would
474 have observed changes in the catabolic profile of the microbial community to guano
475 deposition.

476 For β-glucosidase, acid phosphatase and aryl-sulphatase mass-specific enzyme activities
477 declined with the intensity of guano deposition, suggesting some adjustments in the
478 production of these enzymes in response to increasing availability of C, P and S. In
479 contrast, mass-specific urease activity did not decline with guano deposition. This
480 enzyme showed large increases in activity in the dry season, when maximal rates were >
481 70 times greater than the average value reported for a range of Mediterranean forest
482 soils (Sardans and Peñuelas, 2013). At this sampling time, shortly after the end of the
483 nesting season, accumulation of urea in soils is likely to be at its maximal levels, given
484 the recent deposition of uric acid -the main bird excretion product which decomposition

485 produces urea- and the low soil moisture conditions that minimizes the leaching of urea
486 from soils. Thus, it is likely that microbial urease enzyme production was stimulated in
487 response to increases in the target substrate in the soil environment. It is also possible
488 that urease was not only produced by soil microbes, but also contained in the recently
489 deposited avian excreta, as has been suggested for other bird colonies in Antarctic
490 islands (Speir and Ross, 1984; Tscherko et al., 2003). The large increases in urease
491 activity during the dry season, releasing N-NH_4 as product, together with a possible
492 inhibition of nitrification with salinity (Akhtar et al., 2012), could explain the large
493 accumulation of N-NH_4 observed at the dry season in those soils under a high bird
494 nesting influence.
495

496 4.2. Influence of guano deposition on fungal, bacterial, and archaeal soil communities

497 We hypothesized a decline in fungal richness and diversity at the high end of the guano
498 gradient, in agreement with the known adverse effects of salinization and fertilization
499 on fungal growth and diversity, that often leads to a decrease in fungal dominance
500 (Bradley et al., 2006; Wallestein et al., 2006; Demoling et al., 2008; Rath and Rousk,
501 2015; Rousk et al., 2011a). Indeed, soil fungal community in the different nesting
502 intensity categories was clearly separated in the NMDS ordination at both seasons (Fig.
503 9), and soil P and EOC were the environmental variables with greatest influence on the
504 structure of soil fungal community. However, the total number of TFRs and the fungal
505 diversity index did not change along the studied gradient, and therefore we could not
506 confirm our hypothesis. Terminal restriction fragment analysis does not give any
507 taxonomical information, and therefore we cannot elucidate whether the ordination of
508 the fungal community along the avian intensity gradient is related to changes in the

509 relative abundance of different functional groups. Nevertheless, it is quite likely that the
510 trophic structure of the fungal community was impacted by avian intensity, with
511 decreases in the abundance of mycorrhizal or ligninolytic species and increases in the
512 dominance of coprophilous fungi under a high avian intensity, as found in other areas
513 affected by ornithogenic degradation (Osono et al., 2002; Osono, 2011; Kutorga et al.,
514 2013). Preliminary analyses of the colonization of cork oak roots by ectomycorrhizal
515 fungi suggest a dramatic decline in root-mycorrrhizae associations at the high end of the
516 avian intensity gradient (García et al., unpublished). The distribution of some
517 pathogenic pseudofungi (oomycetes), with an important role in the cork oak population
518 dynamics, seems also to be strongly affected by bird nesting (Serrano et al. 2011).

519 Bacterial TRFs richness and diversity decreased along the gradient of avian intensity in
520 the dry season. In comparison to the wet season, bacterial profiles in the different bird
521 nesting categories were more clearly differentiated in the NMDS analysis, which
522 suggested a key role of soil labile organic C and CE in determining the patterns of
523 bacterial TRFs variability (Fig 9). This suggests that in the dry season, when the bird
524 footprint on soil was at its highest level due to the recent deposition of fresh guano, the
525 environmental conditions imposed by the inputs of avian detritus exerted a selective
526 pressure in the bacterial community. In an African savanna the deposition of vulture
527 guano onto soils imposed a strong habitat filtering for bacterial community structure by
528 selecting for taxa with a higher degree of clustering in phylogeny, which was interpreted
529 as a selection for those taxa with the ability to utilize uric acid and its byproducts as a
530 nitrogen source (Ganz et al., 2012). In addition, the stronger response of the bacterial
531 community to the nesting intensity at the dry season might be related to the greater soil
532 pH variability across nesting categories at this sampling time (with a trend for greater
533 pH under high nesting intensity conditions), given the proposed relationship between

534 bacterial diversity and pH for acidic soils (Fierer and Jackson, 2006) and the apparent
535 stronger sensitivity of bacterial growth to pH changes in comparison to fungi (Rousk et
536 al., 2010).

537 Archaeal diversity showed the clearest response to avian intensity in this study. In the
538 wet season, archaeal TRF H index was significantly higher in the HNI category than in
539 the LNI category (Fig. 8), and positively related to soil P. In the dry season, this index
540 showed a positive significant correlation with the integrated index of avian chemical
541 footprint. Archaea in the Crenarchaeota I.1b clade appear to be the most widely spread
542 and common soil archaea (Timonen and Bomberg, 2009; Bates et al., 2011), which has
543 been shown to have genes encoding enzymes involved in ammonia oxidation (AmoAB,
544 Treusch et al. 2005). Fertilisation of soils with urea has been shown to stimulate
545 archaeal AmoAB genes in acidic soils (Lu et al., 2012). It has been suggested that
546 bacterial and archaeal ammonia oxidizers maintain competitive interactions (Bates et al,
547 2011), and that archaeal ammonia oxidizers exhibit a competitive advantage over
548 bacterial ammonia oxidizers under moderate and high salinity conditions (Zhang et al.,
549 2015). Thus, it is likely that in those soils under a high nesting intensity the high inputs
550 of uric acid, releasing urea during decomposition, promoted the abundance of archaeal
551 ammonia oxidizers, outcompeting their bacterial counterparts due to increased soil
552 salinity. Besides the increases in soil salinity and urea inputs to soil, the concurrent
553 increases in soil P and organic C in those soils fertilized by birds might have also
554 stimulated archaeal ammonia oxidizer proliferation, as found in some fertilization
555 experiments (He et al., 2007). Our results suggests that the archaea community is highly
556 sensitive to the environmental changes studied in this ecosystems, and are in agreement
557 with recent findings reporting a higher sensitivity of archaeal communities than

558 bacterial and fungal communities to disturbances in soils with a long history of N-
559 fertilization (Pereira e Silva et al., 2012).

560 In conclusion, we showed that the presence of a large colony of wading birds in this
561 National Park, promoted by the protection of the area as Natural Reserve, led to strong
562 changes in soil chemical conditions, which impacted soil microbial activity and
563 community composition. Further experimental work is needed to elucidate whether such
564 changes in soil microbial communities have a significant impact on soil C balance in the
565 long term. In any case, the occurrence of detrimental effects of the oversized bird
566 colony on soil chemical conditions, which alter the activity and composition of the
567 native soil microbial communities and threaten the survival of the cork oak population
568 in the area of the colony (García et al., 2011, Fedriani et al., 2016), obligates to a re-
569 evaluation of the management strategies in the area, towards a greater consideration of
570 soil processes in conservation priorities.

571

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877 **Figure Captions**

878

879 Fig. 1. Relationship between electrical conductivity and extractable organic carbon
880 (EOC) across individual soil samples (correlation coefficients and p-values indicated).

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882 Fig. 2. Predicted variation (on a natural logarithmic scale) in soil microbial biomass in
883 response to the bird chemical footprint (mean ± standard error). Used parameters
884 resulted from the generalized linear mixed models reported in Supplementary Material,
885 Table S1. In the dry season the selected model included soil pH as a significant
886 predictors, and microbial C and N under different soil pH conditions (minimum and
887 average pH values) are indicated. Bird chemical footprint index was extracted by a PCA
888 applied to soil chemical variables -see Table 2 for details-. Pseudo R^2 indicates the
889 variance explained by the fixed factors (bird chemical footprint and soil pH).

890

891 Fig. 3. Predicted variation in basal respiration in response to the bird chemical footprint
892 (mean ± standard error), under different soil pH conditions (minimum and average pH
893 values). Used parameters resulted from the generalized linear mixed models reported in
894 Supplementary Material, Table S1. Bird chemical footprint index was extracted by a
895 PCA applied to soil chemical variables -see Table 2 for details-. Pseudo R^2 indicates the
896 variance explained by the fixed factors (bird chemical footprint and soil pH).

897

898 Fig. 4. Predicted variation in extracellular enzyme activities in response the bird
899 chemical footprint (predicted mean ± standard error). Used parameters resulted from the
900 generalized linear mixed models reported in Supplementary Material, Table S1. For

901 those variables for which soil pH was selected as a significant predictor, predictions
902 under different soil pH conditions (minimum and average pH values for each season)
903 are shown. Bird chemical footprint index was extracted by a PCA applied to soil
904 chemical variables -see Table 2 for details-. Pseudo R^2 indicates the variance explained
905 by the fixed factors (bird chemical footprint and soil pH).

906

907 Fig. 5. Diversity (H index) of soil fungal (A), bacterial (B) and archaeal (C) terminal
908 restriction fragments (TRFs) along the nesting intensity gradient. LNI: Low Nesting
909 Intensity; MNI: Medium Nesting Intensity; HNI: High Nesting Intensity.

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911 Fig. 6. Non-metric multidimensional scaling (NMDS) ordination of fungal (A, B),
912 bacterial (C, D) and archaeal (E, F) terminal restriction fragments during the wet and the
913 dry season. Site colors were coded according to nesting intensity categories (LNI = Low
914 Nesting Intensity, MNI = Medium Nesting Intensity, HNI = High Nesting Intensity).
915 Ellipses represent centroid and standard deviation of each category in the two-
916 dimensional ordination plane. Two dimensional stress values were <0.2 (k= 2).
917 Projection of the soil abiotic variables in the ordination plane resulting from the indirect
918 gradient analysis is also indicated. Only those variables with a significant o marginally
919 significant correlation ($p < 0.1$) with ordination axis are shown.

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Table 1. Soil chemical properties across the three bird nesting intensity categories considered (Low, Medium and High) at the wet and dry seasons (mean ± standard deviation of five trees per category, three samples averaged per tree). For each season different letters indicate significant differences among categories (analyzed by linear mixed/ generalized linear mixed models, significance level fixed to $p<0.05$). OM: organic matter; EOC: K_2SO_4-extractable organic C.

	Wet Season			Dry Season		
	Low	Medium	High	Low	Medium	
pH	5.5 ± 0.1 a	5.8 ± 0.2 a	5.6 ± 0.7 a	5.8 ± 0.5 a	6.4 ± 0.8 a	7.0 ± 0.4 b
EC ($\mu S\ cm^{-1}$)	25.1 ± 34.9 a	208.5 ± 174.8 b	497.5 ± 507.3 b	108.2 ± 76.9 a	306.9 ± 232.8 b	1415.7 ± 1076.7 c
$N\text{-}NH_4$ ($mg\ kg^{-1}$)	4.8 ± 2.0 a	15.9 ± 12.4 b	21.6 ± 2.0 b	3.0 ± 2.4 a	15.7 ± 13.6 a	126.3 ± 171.9 b
$N\text{-}NO_3$ ($mg\ kg^{-1}$)	5.4 ± 4.2 a	155.2 ± 164.9 a	245.2 ± 208.5 b	39.3 ± 12.8 a	164.5 ± 236.1 a	988.0 ± 581.1 b
P ($mg\ kg^{-1}$)	6.0 ± 4.2 a	535.0 ± 451.5 b	724.8 ± 332.3 b	12.0 ± 11.1 a	719.4 ± 764.1 b	1254.4 ± 992.8 b
OM (%)	6.2 ± 2.5 a	9.2 ± 5.9 ab	13.8 ± 2.9 b	7.4 ± 2.2 a	9.1 ± 8 a	18.5 ± 9.3 a
EOC ($mg\ kg^{-1}$)	10.8 ± 2.1 a	33.8 ± 26.4 ab	41.8 ± 18.6 b	184.8 ± 32.7 a	650.0 ± 616.1 b	1268.8 ± 506.7 c

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939 Table 2. Factor-variable correlations resulting from the Principal Component Analyses
940 (PCAs) applied to abiotic soil variables (individual soil samples). The variance
941 explained by each factor is indicated.

Variable	Wet season		Dry Season	
	Factor 1 (71 %)	Factor 2 (14 %)	Factor 1 (66 %)	Factor 2 (14 %)
pH	-0.35	0.88	0.53	0.78
EC	0.97	-0.01	0.96	0.05
$N\text{-}NH_4$	0.76	-0.21	0.84	0.36
$N\text{-}NO_3$	0.97	-0.02	0.67	-0.34
P	0.82	0.34	0.90	-0.29
OM	0.87	0.21	0.90	-0.25
EOC	0.97	0.03	0.80	-0.07

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952 Table 3. Summary of the models selected by the stepwise redundancy analysis (RDA) procedure, applied to soil fungal, bacterial and archaeal
953 communities (terminal restriction fragment abundance). AIC, pseudo-F and p values of the selected models are shown.
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	Fungi		Bacteria		Archaea	
	Wet season	Dry Season	Wet season	Dry Season	Wet season	Dry Season
AIC (start model; best model)	(-36.6; -38.8)	(-27.0;-29.2)	(-44.7; -50.2)	(-16.4; -23.5)	(-26.2, -28.8)	(-27.9; -31.4)
Total Inertia	0.42	0.52	0.34	0.58	0.53	0.5
Constrained inertia (%)	7.8	14	14.5	4.5	8.6	7.6
Unconstrained inertia (%)	92.2	86	85.5	95.5	91.4	92.4
Selected variables (F, p)	P (3.62, 0.001)	DOC (2.82, 0.001)	P (3.66, 0.006)	DOC (2.01, 0.057)	P (4.02, 0.001)	DOC (3.51, 0.001)
		pH (1.96, 0.005 P (1.88, 0.005)	MO (3.50, 0.004)			

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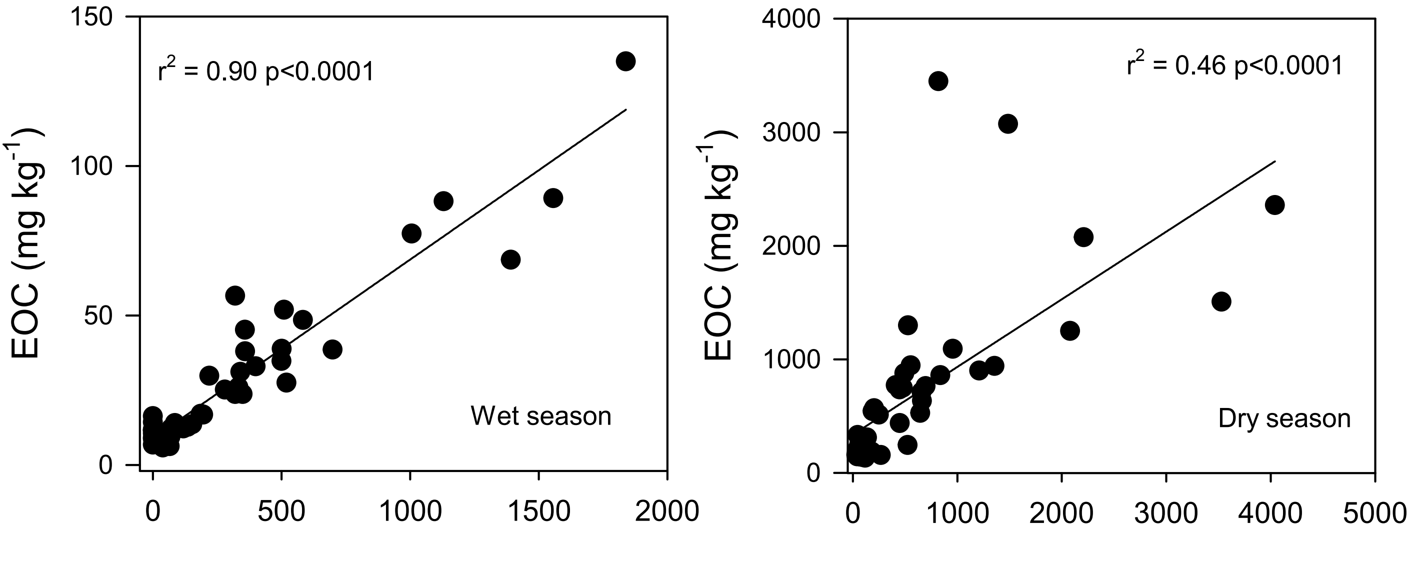
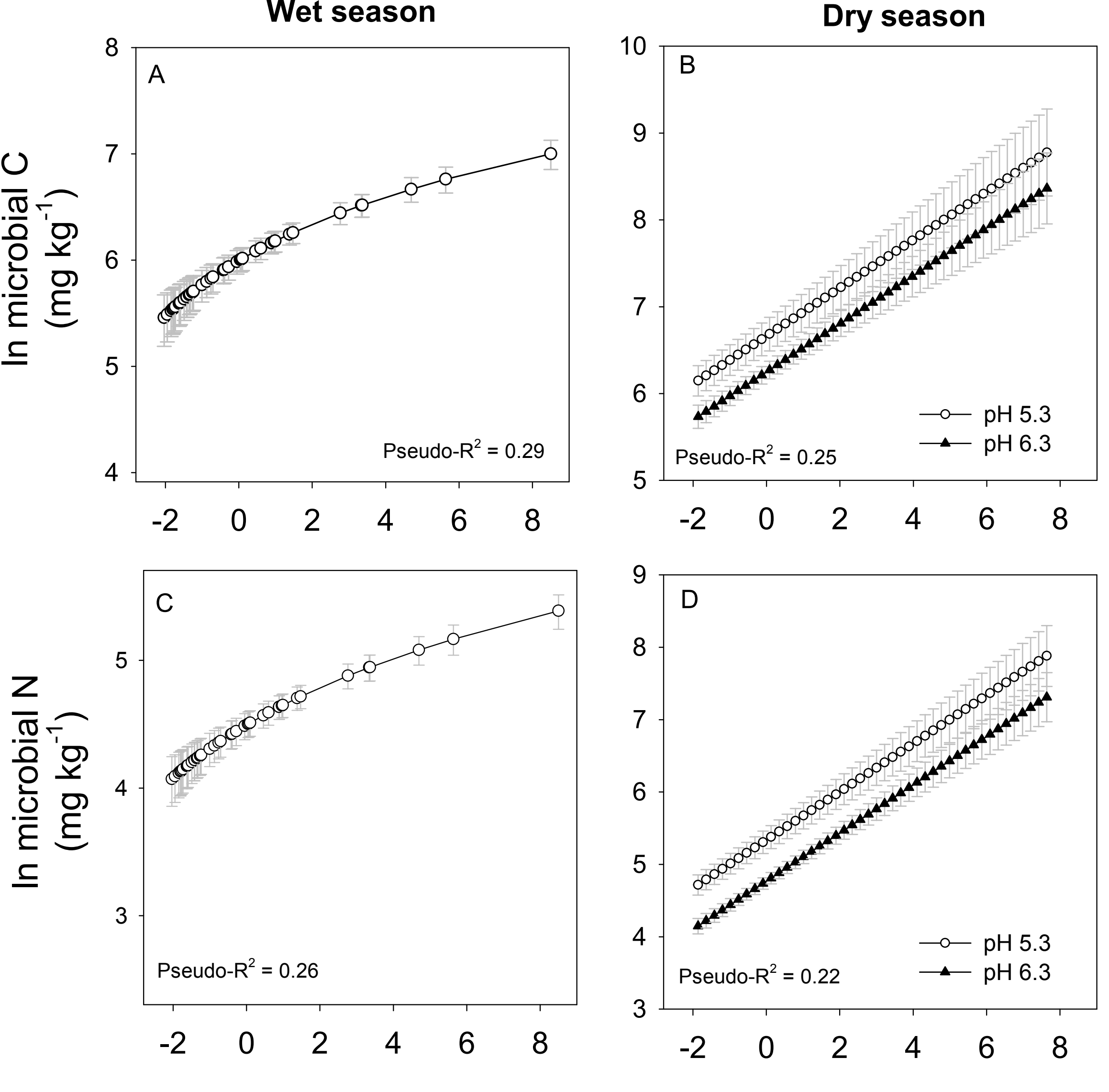
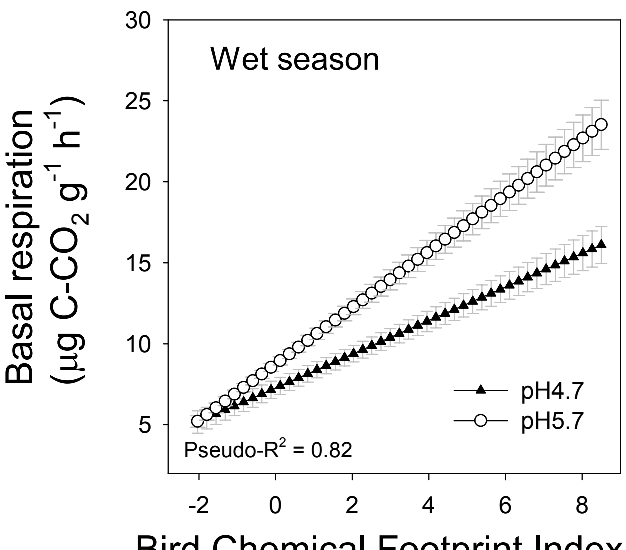


EOC (mg kg-1)
r2 = 0.90 p<0.0001
Wet season
150
100
50
0
0
500
1000
1500
2000
EOC (mg kg-1)
r2 = 0.46 p<0.0001
Dry season
4000
3000
2000
1000
0
0
1000
2000
3000
4000
5000
Electrical conductivity (μS cm-1)



Wet season
Dry season
ln microbial C
(mg kg^{-1})
ln microbial N
(mg kg^{-1})
A
B
C
D
Pseudo-R^2 = 0.29
Pseudo-R^2 = 0.25
Pseudo-R^2 = 0.26
Pseudo-R^2 = 0.22
pH 5.3
pH 6.3
Bird Chemical Footprint Index


Wet season
Basal respiration
(µg C-CO2 g-1 h-1)
30
25
20
15
10
5
pH4.7
pH5.7
Pseudo-R2 = 0.82
-2
0
2
4
6
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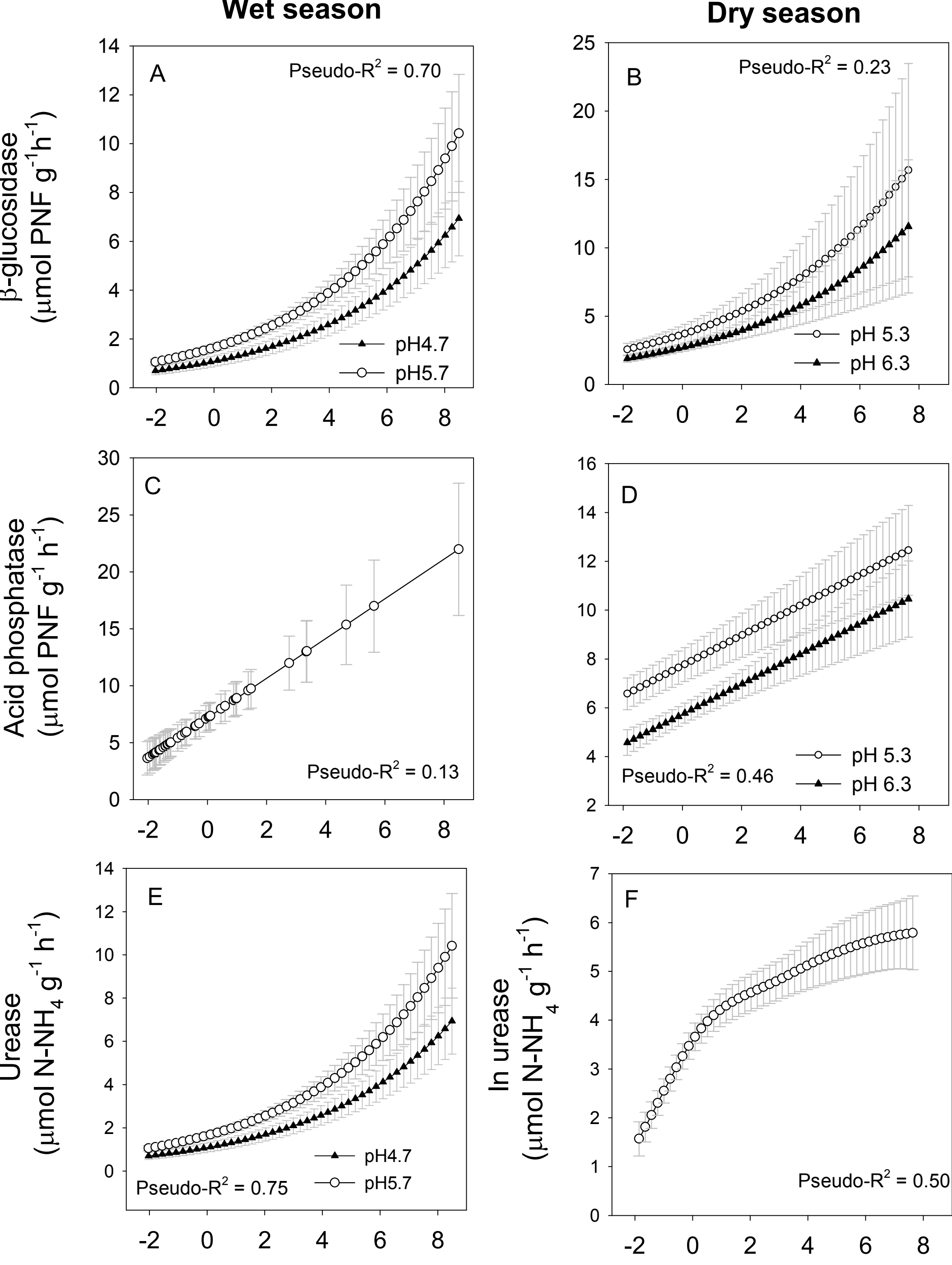
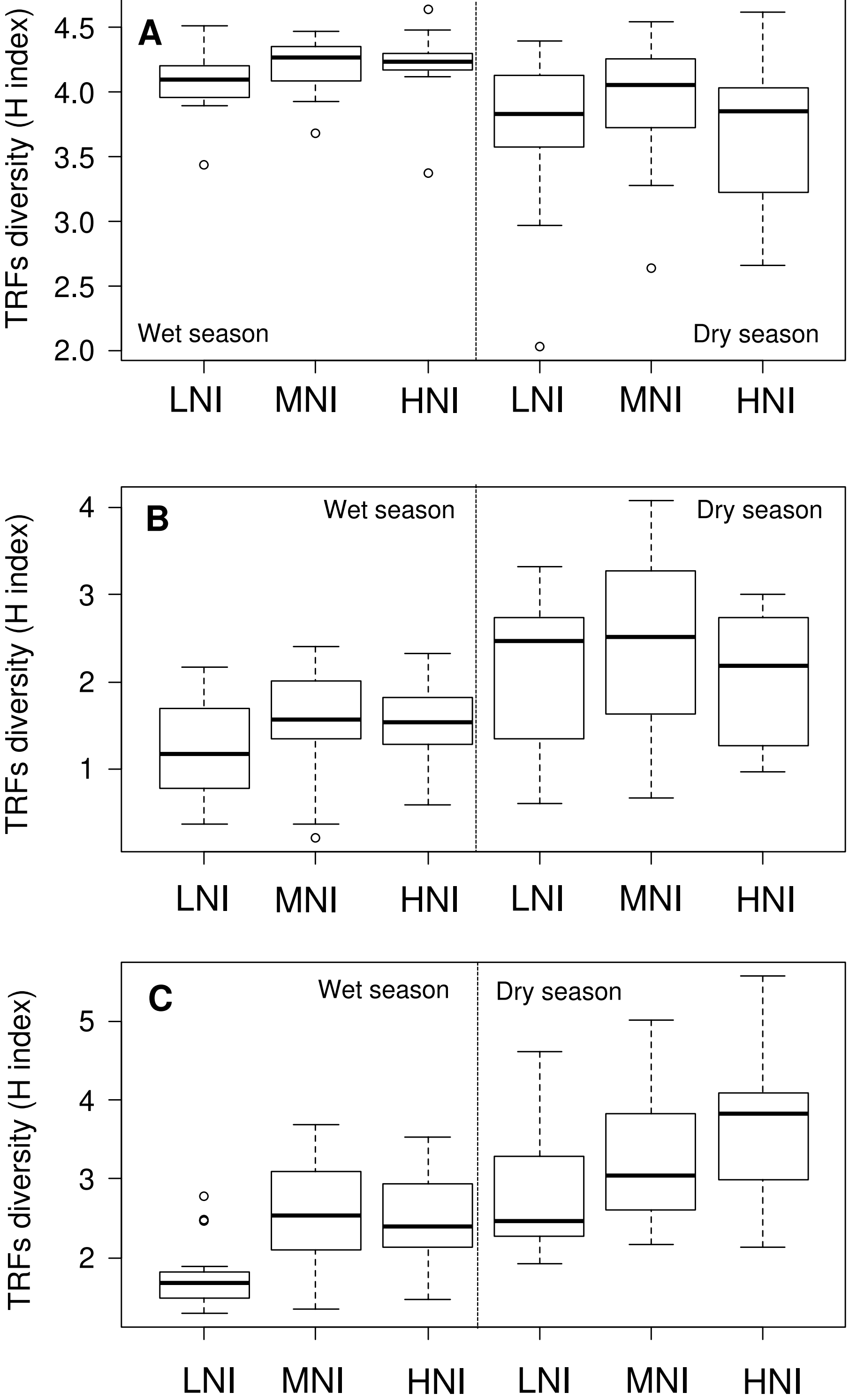
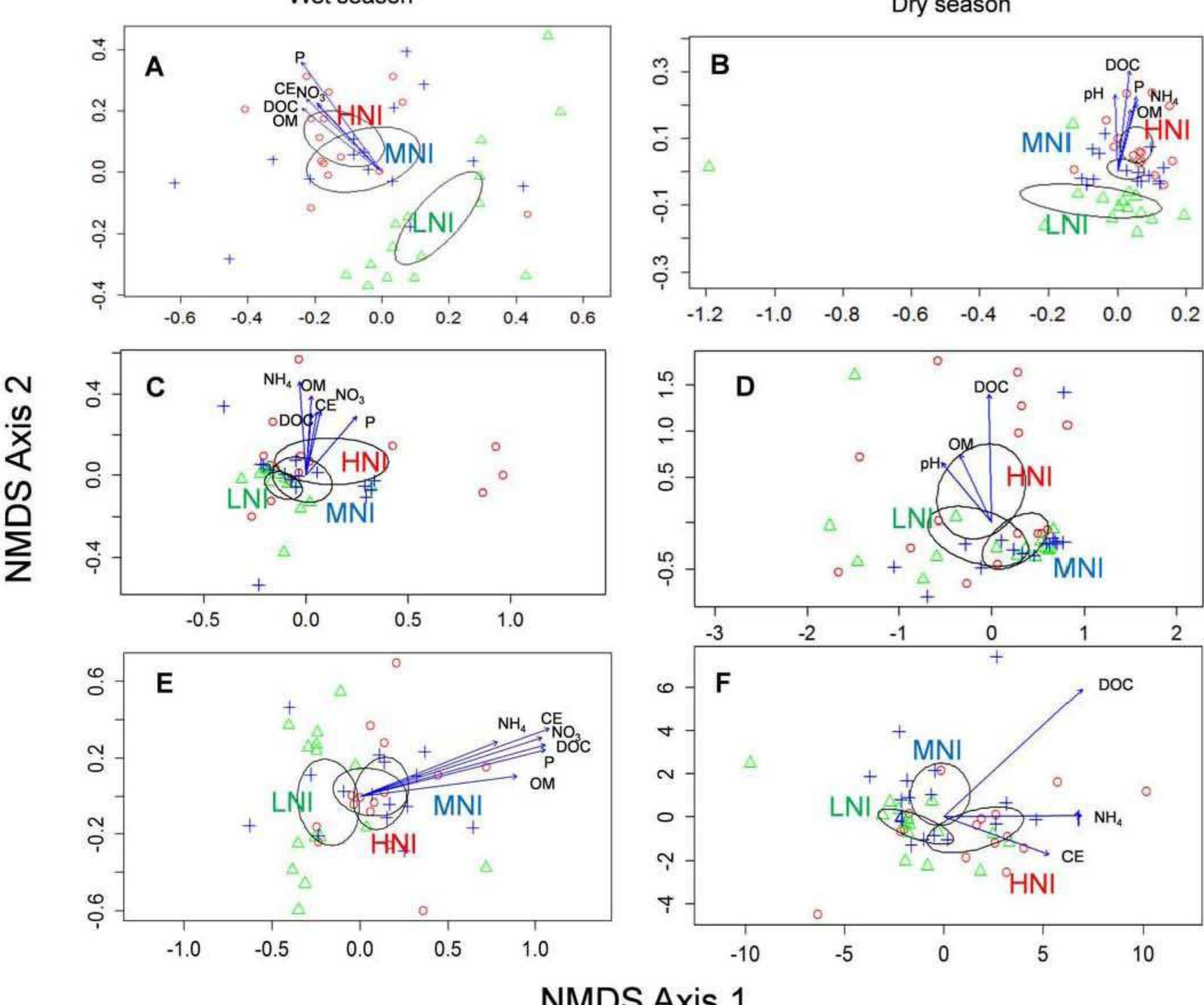
Bird Chemical Footprint Index



Wet season
Dry season
A
Pseudo-R^2 = 0.70
pH4.7
pH5.7
β-glucosidase (μmol PNF $g^{-1} h^{-1}$)
B
Pseudo-R^2 = 0.23
pH 5.3
pH 6.3
C
Pseudo-R^2 = 0.13
Acid phosphatase (μmol PNF $g^{-1} h^{-1}$)
D
Pseudo-R^2 = 0.46
pH 5.3
pH 6.3
E
Urease (μmol N-NH_4 $g^{-1} h^{-1}$)
pH4.7
pH5.7
Pseudo-R^2 = 0.75
F
ln urease (μmol N-NH_4 $g^{-1} h^{-1}$)
Pseudo-R^2 = 0.50
Bird Chemical Footprint Index


A
TRFs diversity (H index)
4.5
4.0
3.5
3.0
2.5
2.0
Wet season
Dry season
LNI
MNI
HNI
LNI
MNI
HNI
B
TRFs diversity (H index)
4
3
2
1
Wet season
Dry season
LNI
MNI
HNI
LNI
MNI
HNI
C
TRFs diversity (H index)
5
4
3
2
Wet season
Dry season
LNI
MNI
HNI
LNI
MNI
HNI


Wet season
Dry season
A
B
C
D
E
F
HNI
MNI
LNI
P
CE
NO3
DOC
OM
NH4
pH
NMDS Axis 2
NMDS Axis 1

Supplementary Material

Title: Impacts of protected colonial birds on soil microbial communities: when protection leads to degradation

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Supplementary Methods: data analysis

We explored the relationships between microbial variables (microbial biomass, enzyme activities, basal respiration, functional diversity and richness and diversity of fungal, bacterial and archaeal TRFs) and a reduced set of soil chemical predictors (the Bird Chemical Footprint Index -BCF- and soil pH) through scatterplot examination, which suggested linear o exponential relationships with BCF for most of microbial variables. LMM o GLMM models were then applied, depending of the distribution of the variables, with BCF and soil pH as fixed factors and tree identity as random factor (see Table S1 for a summary of model types applied). For some variables showing a non-linear relationship with BCF we applied additive mixed models, using the mgcv package in R 3.1.2. For each microbial variable we compared different alternative models: univariate models with each of the selected predictors (pH and BCF) included individually and bivariate models with both BCF and soil pH included either additively or multiplicatively (BCF × pH interaction). Models were compared against a null model, assuming no influence of any of these predictors on soil microbial parameters. Models were selected based on Akaike Information Criteria corrected for small sample size (AICc). The model with the strongest empirical support had the minimum AICc, and pairs of models with ΔAICc between 0 and 2 were considered to have equivalent empirical support (Burnham and Anderson 2002). For each selected model, likelihood-ratio based pseudo-R^2 was calculated based on the improvement from null (intercept only) model, which represents the 'variance explained' by fixed effects (Magee, 1990), using the MuMIn package.

Table S1. Summary of the linear (LMM) or generalized linear (GLMM) mixed models applied to soil microbial variables, with Guano Deposition Index (GDI) or soil pH as predictor variables. Distributions used in GLMMs are indicated. For each variable, only results for the best models (lowest AICc) are shown. qCO_2: metabolic quotient; H-index: index for functional diversity (community level physiological profile), TRFs: terminal restriction fragments.

Variable	Season	Model type	Selected predictors	AICc	Intercept	Predictor estimate	Estimate st. error	Significance (Pr(>|z|))	Pseudo R^2
Microbial C	Wet	GAMM		626	401				0.29
			GDI			180.7	37.7	0.006	
	Dry	GAMM, gamma, log link		79.2	8.85				0.25
			GDI			0.588	0.109	< 0.001	
			pH			-0.414	0.174	0.0102	
Microbial N	Wet	GAMM, gaussian		478	89.45				0.26
			GDI			33.53	7.3	< 0.001	
	Dry	GLMM,Gamma, log link		76.2	8.35				0.32
			GDI			0.715	0.092	< 0.001	
			pH			-0.59	0.125	< 0.001	
Microbial C:N	Wet	LMM		130	10.68				0.16
			pH			-1.1	0.35	0.004	
	Dry	GLMM,Gamma, log link	Null	177	1.53				
Basal Respiration	Wet	LMM		159	0.38				0.82
			GDI			-2.25	1.3	0.094	
			pH			1.46	0.53	0.01	
			GDI × pH			0.7	0.25	0.009	
qCO_2	Wet	GLMM,Gamma, log link		-227	-7.44				0.08
			pH			0.7	0.34	0.044	

Table S1 (continuation)

Variable	Season	Model type	Selected predictors	AICc	Intercept	Predictor estimate	Estimate st. error	Significance (Pr(>|z|))	Pseudo R^2
H index	Wet	GLMM,Gamma, log link	Null	-550	0.3				
Acid phosphatase (pho.)	Wet	LMM		275	7.17				0.12
			GDI			1.74	0.64	0.011	
	Dry	LMM		177	18.27				0.28
			GDI			0.56	0.17	0.005	
			pH			-2	0.51	< 0.001	
Mass-specific pho.	Wet	LMM, log-normal		80.9	3.16				0.13
			pH			-0.11	0.15	0.47	
	Dry	GLMM,Gamma, log link		280	2.43				0.46
			GDI			-0.2	0.06	< 0.001	
Urease (ure.)	Wet	GLMM, log-normal		175	0.78				0.75
			GDI			-1.64	0.38	< 0.001	
			pH			0.06	0.2	0.781	
			GDI × pH			0.36	0.07	< 0.001	
	Dry	GAAM, Gamma, log link		120					0.50
						1.29	0.59	0.038	
Mass-specific ure.	Wet	GLMM,Gamma, log link	Null	269	2.18				
	Dry	GLMM,Gamma, log link		130	-1.26				0.05
			pH			0.8	0.304	0.0128	
Aryl-sulphatase (sul.)	Wet	GLMM, log-normal		-29.8	-1.68				0.16
			GDI			0.17	0.07	0.045	
	Dry	LMM		-44	0.82				0.26
			GDI			0.07	0.02	< 0.001	

Table S1 (continuation)

Variable	Season	Model type	Selected predictors	AICc	Intercept	Predictor estimate	Estimate st. error	Significance $(Pr(>\lvert z\rvert))$	Pseudo R^2
			pH			-0.10	0.04	0.027	
Mass-specific sul.	Wet	GLMM, Gamma, inverse link		62.4	1.59				0.63
			GDI			-3.08	0.81	< 0.001	
			pH			0.06	0.29	0.83	
			GDI × pH			0.64	0.16	< 0.001	
	Dry	GLMM, log-normal		-6.26	-1.79				0.21
			GDI			-0.21	0.08	0.0156	
b-Glucosidase (glu.)	Wet	GLMM, log-normal		102	-1.82				0.7
			GDI			0.21	0.03	< 0.001	
			pH			0.41	0.18	0.023	
	Dry	GLMM,Gamma, log link		134	2.9				0.23
			GDI			0.17	0.05	< 0.001	
			pH			-0.3	0.13	0.024	
Mass-specific glu.	Wet	GLMM,Gamma, log link	Null	222	1.61				
	Dry	GLMM,Gamma, log link		193	1.72				0.42
			GDI			-0.12	0.04	0.009	
Fungal TRFs richness	Wet	GLMM,Gamma, log link	Nulll	493	5.45				
	Dry	LMM	Null	520	194				
Fungal TRFs diversity	Wet	GLMM,Gamma, log link	Nulll	7.3	1.42				
	Dry	GLMM,Gamma, log link	Nulll	7.3	1.42				
Bacterial TRFs richness	Wet	GLMM, log-normal	Nulll	584	4.11				
	Dry	GLMM, log-normal			4.84				0.13
			GDI	678		-0.21	0.09	0.03	
Bacterial TRFs diversity	Wet	GLMM, log-normal	Null	82.5	0.32				
	Dry	GLMM, log-normal		112	0.74				0.24
			GDI			-0.07	0.04	0.062	

Table S1 (continuation)

Variable	Season	Model type	Selected predictors	AICc	Intercept	Predictor estimate	Estimate st. error	Significance (Pr(>|z|))	Pseudo R^2
Archaeal TRFs richness	Wet	GLMM,Gamma, log link	Nulll	443.4	4.28				
	Dry	LMM	Nulll	494	234				
Archaeal TRFs diversity	Wet	GLMM, log-normal	Nulll	77	0.77				
	Dry	LMM		110.75	3.11				0.3
			GDI			0.32	0.11	0.005	

References

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