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

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Abstract

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

nesting largely impacted soil chemical environment, with increases from 25 to 500 $\mu\text{S cm}^{-1}$ 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

- Nesting birds compromised the maintenance of the native soil microbial communities

1. Introduction

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

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

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

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

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

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

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

2. Material and Methods

2.1. Study site

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

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

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

2.2. Sampling design

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

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

In each category five cork oak trees were selected, and soils were sampled underneath the tree cover in three different positions (N, SE and SW) at a 2 m distance from the trunks. In each of these positions, a 1 m² quadrat was established, and three soil samples

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

2.3. Soil processing and chemical analysis

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

Soil pH and salinity (electric conductivity) were measured in 1:5 (soil: water) extracts. Organic matter content was determined by the loss of ignition method, after burning the sample at 540 °C for 3 h. Nitrate and ammonium content in 1M KCl soil extracts were determined spectrophotometrically. Phosphorous was analyzed using the Bray and Kurtz method (Bray and Kurtz, 1945). Organic carbon content in 0.5M K₂SO₄ soil extracts (Extractable Organic C -EOC-) was determined using a Shimadzu TOC-V SCH analyzer.

2.5. Soil microbial biomass and activity

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

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

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

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

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

(Favorgen Biotech Corp, Ping-Tung, Taiwan) and quantified using Quant-iT™ PicoGreen®.

Cleaned PCR products (1500 ng) were digested in a 40 µl reaction mixture consisting of 4 µl of 10 x Tango buffer and a aliquot of HhaI restriction enzyme to a final concentration of 5 U (reagents sourced from Fermentas UAB, Vilnius, Lithuania), during 3 h at 37°C. Products were purified using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany), and quantified again using Quant-iT™ PicoGreen®. After purification, aliquots of each sample were sent to Secugen SL (Madrid, Spain) for analysis of Terminal Restriction fragments (TRFs) on an ABI3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The size of the TRFs was determined using GeneScan™ 500 LIZ® Size Standard (Applied Biosystems, Foster City, CA, USA). TRFs between 50 and 500 base pair length with amplitude greater than 50 fluorescent units were analyzed using Peak Scanner v1.0 (Applied Biosystems, Foster City, CA, USA). Relative abundances were calculated from peak fluorescence values. TRFs data was finally examined and filtered using T-REX software (Culman et al., 2009). For each sample Shannon diversity index was computed from fungal, bacterial and archaeal TRFs, as described above.

2.7. Data analysis

Data analysis was conducted using different packages in R 3.1.2. Linear or generalized linear mixed models were applied after exploration of frequency distributions of soil chemical variables to test for differences among nesting intensity categories, using the nlme (mixed linear models, LMM) or the lme4 (generalized linear mixed models, GLMM) packages. Nesting intensity was set as fixed factor, and tree identity as random

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

Heterogeneity in soil chemical properties within bird nesting categories and among samples collected underneath individual trees was explored by calculating coefficients of variation. The high heterogeneity of soil salinity and nutrients within the MNI and HNI categories suggested a large variability in the impact of our a-priori variable (historical nesting intensity recorded at each sample tree) on soils. In order to explore changes in soil microbial activity and community structure along a continuous gradient of bird influence, rather than among nesting intensity categories, we conducted a Principal Component Analysis (PCA) of soil abiotic variables (individual samples) for each season, to extract an integrated continuous index of avian influence for each datasets. For both datasets the first PCA component integrated the main soil chemical gradient (Table 1), and was used thereafter as a Bird Chemical Footprint (BCF) index. To test our hypotheses about the impact of the bird influence on soil microbial communities we also used this quantitative index, and explored the shape of 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 integrated BCF and soil pH) through a modelling approach, applying LMM, GLMM or additive mixed models (see details of the modelling approach in Supplementary Information).

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

abundance matrices were performed using Non-metric Multidimensional Scaling (NMDS). The rankindex function in the vegan package was previously applied to each data set to evaluate which of the distance indices best separated communities along the environmental gradient considered (bird influence intensity), using rank correlations. Euclidean distance was used for all data sets, except for bacterial and archaeal communities in the dry season, for which Bray-Curtis and Manhattan distances were used, respectively. Environmental vectors representing the studied soil abiotic variables were fitted onto the ordination planes using the envfit function. Then, constrained analysis (Redundancy Analysis, RDA) was applied to study the variation in TRFs composition due to the main soil chemical gradient (BCF) and soil pH, with the block factor (tree identity) introduced as conditional factor. We further conducted the RDA analyses with a subset of individual soil chemical variables, instead of the integrated index: pH, CE, N-NH₄, P, and organic matter (wet season) or extractable organic C (dry season). The best combination of explaining variables was selected by applying a forward model building procedure. Variance inflation factors were calculated for the final models, to check for independency of the selected predictors.

3. Results

3. 1. Soil chemistry along the nesting intensity gradient

Nesting intensity did not have a significant influence on soil pH (Fig. 1a). Electrical conductivity (EC), however, was highly influenced by bird nesting, being average EC values 8 and 20 times higher in the MNI and HNI categories, respectively, in comparison to the LNI category during the wet season (Table 1). Salinity increased 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- $\text{NH}_4 \text{ kg}^{-1}$ for the MNI and the
322 HNI categories, respectively. Nitrate followed an opposite pattern, soil concentrations in
323 the wet season being greater than 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

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

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

3.2. Soil microbial biomass and activity response to guano deposition

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

Soil basal respiration ranged from 5 to $19 \mu\text{g C-CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ between the extremes of the guano deposition gradient. The response of soil basal respiration to BCF also depended on soil pH, being more stimulated with increasing pH conditions (Fig. 3). In contrast,

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

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

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

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

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

4. Discussion

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

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

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

Indeed, in our study we detected increases of >10 times in soil EC_{1:5}, > 100 times in soil P and 3-45 times (depending on the season) in N-NH₄ and N-NO₃ between the extremes of the avian intensity gradient. At the dry season we recorded soil EC_{1:5} values as high as 4000 $\mu\text{S cm}^{-1}$. Similar increases in soil salinity have been reported to inhibit microbial growth (Rousk et al., 2011b), decrease microbial biomass (Sardinha et al., 2003; Tripahi et al., 2006), provoke a decline in soil respiration (Setia et al., 2011; Rath and Rousk, 2015,) and impair extracellular enzyme production (García and Hernández 1996; Saviozzi et al., 2011) in a range on studies with different soil types. However, and in contrast to our hypothesis, we observed positive linear or log-linear relationships between the bird nesting footprint on soils (indicated by the integrated avian intensity index) and microbial biomass, basal respiration and most of the studied enzyme activities. Interestingly, the magnitude of the stimulation of microbial C and N and by bird inputs was dependent on soil pH, remaining greater at pH values around 5.6 than at pH above 6.3 (seasonal median) during the dry season. This suggests that pH conditions close to 5.5 are optimal for microbial growth in these naturally-acidic forest soils.

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

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

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

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

produces urea- and the low soil moisture conditions that minimizes the leaching of urea from soils. Thus, it is likely that microbial urease enzyme production was stimulated in response to increases in the target substrate in the soil environment. It is also possible that urease was not only produced by soil microbes, but also contained in the recently deposited avian excreta, as has been suggested for other bird colonies in Antarctic islands (Speir and Ross, 1984; Tscherko et al., 2003). The large increases in urease activity during the dry season, releasing N-NH₄ as product, together with a possible inhibition of nitrification with salinity (Akhtar et al., 2012), could explain the large accumulation of N-NH₄ observed at the dry season in those soils under a high bird nesting influence.

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

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

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

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

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

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

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

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

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Figure Captions

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

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

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

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

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

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

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

Table 1. Soil chemical properties across the three bird nesting intensity categories considered (Low, Medium and High) at the wet and dry seasons (mean $\pm$ 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 $\pm$ 0.1 a	5.8 $\pm$ 0.2 a	5.6 $\pm$ 0.7 a	5.8 $\pm$ 0.5 a	6.4 $\pm$ 0.8 a	7.0 $\pm$ 0.4 b
EC ($\mu S\ cm^{-1}$)	25.1 $\pm$ 34.9 a	208.5 $\pm$ 174.8 b	497.5 $\pm$ 507.3 b	108.2 $\pm$ 76.9 a	306.9 $\pm$ 232.8 b	1415.7 $\pm$ 1076.7 c
N-NH ₄ (mg kg ⁻¹)	4.8 $\pm$ 2.0 a	15.9 $\pm$ 12.4 b	21.6 $\pm$ 2.0 b	3.0 $\pm$ 2.4 a	15.7 $\pm$ 13.6 a	126.3 $\pm$ 171.9 b
N-NO ₃ (mg kg ⁻¹)	5.4 $\pm$ 4.2 a	155.2 $\pm$ 164.9 a	245.2 $\pm$ 208.5 b	39.3 $\pm$ 12.8 a	164.5 $\pm$ 236.1 a	988.0 $\pm$ 581.1 b
P (mg kg ⁻¹)	6.0 $\pm$ 4.2 a	535.0 $\pm$ 451.5 b	724.8 $\pm$ 332.3 b	12.0 $\pm$ 11.1 a	719.4 $\pm$ 764.1 b	1254.4 $\pm$ 992.8 b
OM (%)	6.2 $\pm$ 2.5 a	9.2 $\pm$ 5.9 ab	13.8 $\pm$ 2.9 b	7.4 $\pm$ 2.2 a	9.1 $\pm$ 8 a	18.5 $\pm$ 9.3 a
EOC (mg kg ⁻¹)	10.8 $\pm$ 2.1 a	33.8 $\pm$ 26.4 ab	41.8 $\pm$ 18.6 b	184.8 $\pm$ 32.7 a	650.0 $\pm$ 616.1 b	1268.8 $\pm$ 506.7 c

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Table 2. Factor-variable correlations resulting from the Principal Component Analyses (PCAs) applied to abiotic soil variables (individual soil samples). The variance 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-NH ₄	0.76	-0.21	0.84	0.36
N-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

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)	MO (3.50, 0.004)			
		P (1.88, 0.005)				

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Electrical conductivity ($\mu\text{S cm}^{-1}$)

Wet season

Dry season

Bird Chemical Footprint Index

Wet season

Dry season

Bird Chemical Footprint Index

NMDS Axis 2

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 or exponential relationships with BCF for most of microbial variables. LMM or 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 $\times$ 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 Δ AICc 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. qCO₂: 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
	Dry	LMM	GDI	177	18.27	1.74	0.64	0.011	0.28
			GDI			0.56	0.17	0.005	
			pH			-2	0.51	< 0.001	
Mass-specific pho.	Wet	LMM, log-normal	pH	80.9	3.16	-0.11	0.15	0.47	0.13
	Dry	GLMM,Gamma, log link	GDI	280	2.43	-0.2	0.06	< 0.001	0.46
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		1.29	0.59	0.038	0.50
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(> z))	Pseudo R ²
Mass-specific sul.	Wet	GLMM, Gamma, inverse link	pH	62.4	1.59	-0.10	0.04	0.027	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	GDI	-6.26	-1.79	-0.21	0.08	0.0156	0.21
b-Glucosidase (glu.)	Wet	GLMM, log-normal		102	-1.82				0.7
			GDI			0.21	0.03	< 0.001	
	Dry	GLMM, Gamma, log link	pH	134	2.9	0.41	0.18	0.023	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				0.42
	Dry	GLMM, Gamma, log link		193	1.72				
			GDI			-0.12	0.04	0.009	
Fungal TRFs richness	Wet	GLMM, Gamma, log link	Null	493	5.45				
	Dry	LMM	Null	520	194				
Fungal TRFs diversity	Wet	GLMM, Gamma, log link	Null	7.3	1.42				
	Dry	GLMM, Gamma, log link	Null	7.3	1.42				
Bacterial TRFs richness	Wet	GLMM, log-normal	Null	584	4.11				0.13
	Dry	GLMM, log-normal			4.84				
			GDI	678		-0.21	0.09	0.03	
Bacterial TRFs diversity	Wet	GLMM, log-normal	Null	82.5	0.32				0.24
	Dry	GLMM, log-normal		112	0.74				
			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	

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