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1 **Biotic and abiotic predictors of potential N₂O emissions from denitrification in Irish**
2 **grasslands soils: a national-scale field study**

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27 **Highlights**

- 28 • Soils with highest nitrification also had the highest denitrification potential (PD).
- 29 • Soil phosphorus was as important predicting PD as was pH and total nitrogen.
- 30 • The prokaryotic community was more predictive of soil PD than the fungal
31 community.
- 32 • Microbial community structure, rather than gene abundance, was an important
33 predictor.
- 34 • Actinobacteriota and Crenarchaeota were correlated to complete denitrification.

35

36 **Abstract**

37 Large-scale information regarding nitrous oxide (N₂O) emissions is needed as an evidence
38 base to underpin land use policy and mitigation approaches. However, the highly variable
39 rates of denitrification make the prediction of N₂O emission demanding. Here, we evaluated
40 the role of abiotic and biotic factors on the potential denitrification of Irish soils, in order to
41 identify the key factors regulating potential N₂O emissions at a large scale. To do so, we
42 collected 136 soil samples from 32 sites across Ireland, and characterised the soil physico-
43 chemical properties, the prokaryotic and fungal community composition, the abundance of N-
44 cycling genes and evaluated the soil potential nitrification, denitrification and end product
45 N₂O / (N₂O + N₂). We found large differences in soil potential denitrification between sites
46 (up to 41.5 mg N₂O-N kg⁻¹ soil day⁻¹) with most of the emissions released in the form of N₂O
47 rather than N₂. Soils with highest potential nitrification rates also exhibited the highest
48 potential denitrification rates, and similar parameters were linked to both processes. The
49 factors most predictive of soil potential denitrification were soil physico-chemical properties
50 and the prokaryotic community composition. Soil phosphorus content was as important for
51 predicting potential denitrification as was pH and total nitrogen. Soil microbial community
52 structure, rather than denitrifier abundance, was an important predictor of the potential
53 denitrification and the end-product N₂O / (N₂O + N₂). The prokaryotic community
54 composition was more strongly associated with denitrification rates and the resulting end-
55 products than fungal communities. Increased relative abundance of the prokaryotic phyla
56 Actinobacteriota and Crenarchaeota, were positively correlated to complete denitrification.
57 Altogether, these results lay the foundation for a better understanding of the key factors

58 regulating the potential denitrification in soils and identify important properties that enhance
59 prediction of the potential denitrification at larger scales.

60

61 **Keywords:** denitrification, nitrification, functional N cycling communities, microbial
62 community structure, nitrous oxide, managed grasslands

63

64 **Introduction**

65 Nitrous oxide (N₂O) is amongst the most important greenhouse gases (GHG), contributing to
66 around 10% of annual global warming, with a global warming potential approximately 265 to
67 298 times greater than CO₂ and an average life-time of 116 years (IPCC, 2021). It is also of
68 concern with respect to its role in ozone layer depletion (Ravishankara et al., 2009).
69 Currently, atmospheric concentrations of N₂O continue to rise, with N₂O reaching 332 ppb in
70 2019, an increase of 8 ppb since 2011 (IPCC, 2021). At a global scale, the primary human
71 activity responsible for the increased N₂O release into the atmosphere is the intensive use of
72 Nitrogen (N) fertilizer in agricultural lands (Tian et al., 2020). In Ireland, agriculture is the
73 largest contributor to GHG emissions, representing 37.1 % of the total emissions, and
74 contributing 92.5 % of the N₂O emissions nationwide (Environmental Protection Agency,
75 2021). It is important to understand the processes leading to the loss of N in a gaseous form,
76 not only as a mechanism to mitigate GHG emissions but also due to the critical role N-cycling
77 plays in the delivery of a range of essential ecosystem services, e.g., plant productivity,
78 nutrient cycling, and soil carbon sequestration (Jones et al., 2014). An enhanced
79 understanding of these processes would facilitate the optimisation of fertiliser applications,
80 with resulting reductions of nutrient losses through the air and leaching. In order to inform
81 land management advice aimed at reducing such emissions, it is crucial to identify the key
82 factors influencing the potential of agricultural soils to release N in the form of N₂O.

83 The major soil processes resulting in N₂O emissions are due to microbial activity via two
84 successional processes called nitrification and denitrification. Both processes are critical steps
85 in the N-cycle, supplying organisms with this rarely bioavailable and growth-limiting nutrient
86 in many environments (Kuypers et al., 2018). The former is the stepwise oxidation of
87 ammonium (NH₄⁺) into nitrite (NO₂⁻), which is driven by ammonia-oxidizing bacteria (AOB)
88 and archaea (AOA); and the subsequent conversion of nitrite to nitrate (NO₃⁻) by nitrite

89 oxidising bacteria. However, the process can also be undertaken by complete ammonia-
90 oxidizing bacteria (comammox) that are able to sequentially oxidize ammonia to nitrate via
91 nitrite in one single cell (Daims et al., 2015; van Kessel et al., 2015). Ammonia oxidizers can
92 also produce N₂O through the oxidation of hydroxylamine during NO₂⁻ formation, where the
93 by-product nitric oxide (NO) can then be oxidized to N₂O via NO reductases (Tierling &
94 Kuhlmann, 2018; Wrage-Mönnig et al., 2018). Denitrification, the main process of N₂O
95 formation (Harris et al., 2021; Mosier, 1998), is a facultative anaerobic process resulting in
96 the stepwise reduction of nitrate into either the environmentally harmful gas N₂O or, in case
97 of complete denitrification, into the benign dinitrogen (N₂) gas (Groffman, 2012). The key
98 step of denitrification is the reduction of soluble nitrogen (nitrite) into a gaseous form (NO)
99 catalysed by the nitrite reductase encoded either by the *nirK* or *nirS* genes. The last step of the
100 denitrification is catalysed by the N₂O reductase encoded by the *nosZ* gene, which is the only
101 known sink of N₂O in the biosphere, as it reduces N₂O into N₂ (Hallin et al., 2018).

102 The capacity to nitrify and denitrify is distinctively distributed among microorganisms, with
103 nitrification being exclusively performed by prokaryotes, while denitrification is present in
104 both prokaryotes and eukaryotes, and carried out by phylogenetically diverse communities
105 within the domains of Archaea, Bacteria and Fungi (Maeda et al., 2015; Philippot et al.,
106 2007). These are modular processes with not all organisms contributing to every step, and
107 thus the soil microbial community structure and abundance are expected to determine N₂O
108 released from soils. Some soil organisms, such as Fungi and Actinobacteria, produce only
109 N₂O because they lack N₂O reductase (Jones et al., 2014; Maeda et al., 2017; Sun et al., 2016;
110 Zhao et al., 2019), while others are only N₂O reducers (Hallin et al., 2018). Further, some
111 organisms such as ammonia oxidizers can also be a source of N₂O emissions under certain
112 edaphic and climatic conditions (Bateman & Baggs, 2005; Khalil et al., 2004). Therefore, the
113 relative proportion of the different denitrifiers and nitrifiers may impact the end-products of
114 soil N-cycling and the resulting potential N₂O emitted. The quantification of functional genes
115 in soils is used as a proxy of the abundances of nitrifiers and denitrifiers but relationships
116 between microbial genes and soil denitrification are not always observed because other key
117 factors interact and influence enzymes activities and the release of N₂O and N₂ in the
118 atmosphere (e.g., Liu et al., 2013).

119 Denitrification processes are regulated by environmental conditions, including soil physico-
120 chemical properties. Numerous local experiments have demonstrated that denitrification is
121 influenced by edaphic conditions such as soil pH, soil moisture and temperature, soil aeration,

122 water-filled pore space and soil carbon (e.g., Brenzinger et al., 2017; Mehnaz et al., 2019;
123 Norton & Ouyang, 2019; Žurovec et al., 2021). Soil physico-chemical properties may also
124 indirectly influence the N₂O emissions via shaping of soil microbial communities involved in
125 N-processes (Samad et al., 2016). Therefore, interactions between biotic and abiotic soil
126 properties rather than individual factors are expected to influence microbial traits and their
127 activity (Graham et al., 2014), and the identification of key predictors is needed to implement
128 more accurate predictive models.

129 Despite the known effects of soil physico-chemical properties and implications of microbial
130 activity in denitrification, N₂O emissions remain highly variable and therefore difficult to
131 predict in grasslands (de Klein et al., 2014). More studies integrating biotic and abiotic factors
132 are needed to understand their relative contribution in driving N₂O emissions. One approach
133 to address these aspects is studying natural gradients to understand the key factors influencing
134 N₂O emissions (Almaraz et al., 2020). Using this approach, both the soil physical properties
135 and microbial community composition were identified as the main drivers of potential
136 denitrification in New Zealand (Morales et al., 2015), while Kou et al., (2019) showed that
137 soil physico-chemical conditions such as pH and total organic carbon, were more important
138 than the abundance of functional genes involved in denitrification. These contrasting results
139 highlight that drivers of potential denitrification vary, and that our understanding of the
140 potential of agricultural soils to release N₂O and capacity to extrapolate this to a broader
141 geographical scale, is still limited.

142 Here, we evaluated the range of potential N₂O emissions from denitrification in Irish
143 grassland soils and evaluated the drivers associated with such processes. The objective was to
144 identify the key factors regulating these processes at a large scale, with the aim of
145 contributing to the broader understanding of the factors with the greatest impacts on N₂O
146 emissions at a global scale. To respond to this objective, we assessed the soil potential
147 denitrification and N₂O emissions in relation to total emissions (N₂O + N₂) from soils
148 collected in thirty-two sites across Ireland. We also evaluated the relationships between these
149 processes and climatic conditions, soil physico-chemical properties, nitrifier and denitrifier
150 abundances as well as prokaryotic (bacteria and archaea) and fungal community structure. We
151 additionally evaluated soil potential nitrification as an important driver of N-availability in
152 soils including forms of N that act as the substrate of denitrification.

153

154 **Materials and Methods**

155 **Sampling sites**

156 To evaluate soil potential denitrification in Irish grasslands we collected a total of 136 soil
157 samples from 32 sites across the island of Ireland (Fig. 1). The closest sites were within 15
158 km, while the furthest were at approximately 500 km distance, and sites were chosen to
159 encompass a wide range of geoclimatic variables and soil physico-chemical properties.
160 Climatic conditions for each site were retrieved from WorldClim2 database using a 1 km²
161 resolution (Fick & Hijmans, 2017). All samples were collected on mineral soils from
162 managed grasslands, the great majority of which were dominated by ryegrass (*Lolium* sp.).
163 Descriptions of the land management are in Table S1, but briefly, paddocks were grazed by
164 cattle or sheep with stocking rates ranging overall from 1 to 2.9 livestock units per hectare.
165 The most recent fertiliser application was a minimum of ten weeks prior to soil sampling.

166

167 **Soil sample collection**

168 Soil samples were collected during the winter period, between mid-November 2019 and early
169 March 2020. Based on previous studies and input from land managers, between three and
170 twelve paddocks were selected for sampling per site in order to incorporate the desired
171 diversity of soil types and a wide range of edaphic properties. Within each paddock, we
172 established a 1 m x 1 m plot from which soil for all subsequent analyses was collected. This
173 approach was used to reduce the spatial variability in prokaryotic and fungal communities,
174 and to better relate soil microbial communities to the soil physico-chemical properties and
175 potential activities.

176 For each plot, three different soil samples were collected and processed separately to
177 characterise 1) the soil physico-chemical properties, and potential nitrification and
178 denitrification, 2) the bulk density, and 3) the soil microbial communities, including nitrifiers
179 and denitrifiers functional gene abundance and overall community composition. For soil
180 physico-chemical characterisation and for both nitrification and denitrification assays, we
181 collected approximately 1 kg of soil from the top 10 cm of the soil surface using an auger.
182 Soil samples were preserved at 4 °C until further processing and analyses were performed. To
183 evaluate bulk density, three samples were taken within the top 10 cm of the soil surface using
184 a 94.28 cm³ core sampling ring and stored at 4°C until drying. Finally, to later characterise the

185 soil microbial communities, five soil cores of 10 cm depth were collected from each corner
186 and the centre of the plot and then pooled as one composite sample. This composite soil
187 sample was flash frozen on site using liquid N and then stored at -80°C until molecular
188 analyses were performed.

189

190 **Soil physico-chemical characterisation**

191 Soil water content and water holding capacity were determined gravimetrically by overnight
192 drying of fresh and water-saturated soils at 105°C (Brischke & Wegener, 2019). 2M KCl was
193 used to extract mineral-N (nitrate-N (NO^{-3}) and ammonium-N (NH^{+4})) from fresh soil
194 (20g). Extracts were then measured through the Aquakem 600 analyzer (Thermo Scientific,
195 USA). Soil available phosphorus (Mehlich III), phosphorous, potassium and magnesium
196 (Morgans), copper (EDTA), sulphur (Calcium Phosphate), total carbon, inorganic C and total
197 N (LECO Trumac CN, Michigan, USA), pH (1/2.5 v/v soil/water), cation exchange capacity
198 (extracted using buffered Barium Chloride with triethanolamine following Avery and
199 Bascomb laboratory methods, 1974), and particle size (sedimentation) were determined. All
200 analyses were performed on dried and sieved soil (at 30°C , 2 mm mesh size), except for total
201 carbon and total nitrogen content, analyses were performed on 150 μm ball-milled dried soil.
202 Dry bulk density was determined following the ISO 11272:1998 procedure (Walter et al.,
203 2016). Concisely, soil cores were dried at 105°C for 48h and dry weight was recorded before
204 sieving (2mm sieve), and the dry weight as well as the volume of $> 2\text{mm}$ stones was
205 determined. Bulk density was calculated as the core dry weight divided by the core volume
206 after accounting the weight and volume of stones (Walter et al., 2016).

207

208 **Soil potential nitrification**

209 Potential nitrification was evaluated within a maximum of a week of sampling following
210 Drury et al. (2008) procedure. Briefly, 15g of sieved fresh soil (4mm mesh) was weighed into
211 a 500 mL schott bottle and 100 mL of freshly prepared NH_4^{+} nutrient solution pH 7.2 (1.5
212 mM NH_4^{+} and 1mM PO_4^{3-}) was added to the soil. Soils were incubated under oxic conditions
213 at 15°C and shaken at 130 rpm for 24h (Wiseshaker SHO-2D, Wisd Lab instruments).
214 Subsamples were taken after 2h, 4h, 21h and 24h of nutrient addition. Filtrates were analysed
215 in the Aquakem 600A Photometric analyser (Thermo Scientific, USA). Nitrification rate for
216 each sample was calculated as described by Drury et al., (2008).

217 **Soil potential denitrification and N₂O production**

218 Soil potential denitrification was assessed in order to determine the maximum rate of
219 nitrogenous gas released from soils under optimal conditions (i.e. anoxic environment, no
220 nutrient limitations, and optimal moisture content). This was evaluated within a week of
221 sampling by using the acetylene blocking method described by Yoshinari et al., (1977) with
222 some modifications. Briefly, for each sample, two 160 mL glass flasks containing 20 g of
223 previously sieved fresh soil (4mm mesh) were sealed and pre-incubated at 15 °C overnight.
224 After the pre-incubation period, moisture content was adjusted to 70% water holding capacity
225 and the headspace was flushed with helium to maintain an anoxic environment. For each
226 sample, one flask was injected with acetylene (C₂H₂; 10% v/v of the headspace) to inhibit the
227 reduction of N₂O to N₂, and then be able to calculate the total N-gas formed through
228 denitrification that could potentially be realised as N₂O. Acetylene was injected into the flasks
229 at the start of the incubation (Time 0). The second flask was injected with helium to allow the
230 whole denitrification pathway to occur. Subsequently, a solution of 75mM KNO₃, 37.5 mM
231 Na-succinate, 25mM glucose and 75mM Na-acetate was added to each flask adjusting the
232 respective volumes to reach 70% of soil's water holding capacity. The flasks were incubated
233 for 5 hours in the dark at 15°C and shaken at 130 rpm (Orbital Platform Shaker PSU-10i,
234 Grant Bio VWR). Gas samples were taken every hour after nutrient solution addition by
235 transferring 15 mL gas from the headspace into a 12mL pre-evacuated gas vial that were then
236 analysed for N₂O concentrations using a 450 Gas Chromatograph with a 63Ni electron
237 capture detector (Bruker, Germany) coupled to a Combi-PAL xt® auto-sampler (CTC
238 Analytics AG, Switzerland). For each flask, the potential N₂O produced through
239 denitrification was estimated as follows:

$$240 \quad \text{Potential N}_2\text{O (ng N}_2\text{O-N/g soil/min)} = \left[\frac{\left(\frac{P * h}{R * T} \right) * m}{W} \right] * \frac{28g}{mol} * 1000$$

241 Where P is the atmospheric pressure, h is the headspace volume, R is the universal gas
242 constant, T is the temperature, m is the rate of N₂O production per minute and W is soil dry
243 weight. Potential denitrification was estimated as the N₂O produced in the acetylene flask, and
244 the N₂O / (N₂O + N₂) ratio was estimated as the potential of soils to emit N₂O over the total
245 gases and was evaluated as N₂O produced in the helium flask over N₂O produced in the
246 acetylene flask.

247

248 **Soil microbial community's characterisation**

249 *DNA extractions from soil and amplicon sequencing*

250 DNA was extracted from 0.25 g soil using the DNeasy PowerSoil Kit (Qiagen, Hilden
251 Germany) following the manufacturer's instructions. The yield and quality of DNA extracts
252 were verified by Qubit dsDNA BR Assay kit using an Invitrogen Qubit 3.0 fluorometer
253 (Thermo Fisher Scientific, USA), a Nanodrop 2000 spectrophotometer (Thermo Fisher
254 Scientific, USA) and by running an 1% agarose gel. Extracted DNA was stored at -80°C until
255 further analysis.

256 Sequencing libraries were prepared for the V4 region of archaeal and bacterial *16S rRNA* gene
257 using the 515F and 926R primers (Parada et al., 2016) and for the fungal ITS2 region,
258 targeted with the 4R and 86F primers (De Beeck et al., 2014). The first PCR was performed
259 with the above-mentioned specific primers flanking Illumina overhang adapters sequences in
260 a 25µL PCR reaction containing 2x KAPA HiFi HotStart ReadyMix (Kapa Biosystems Inc,
261 USA), 1µM of each primer and 2.5µL of DNA. For the *16S rRNA* gene, the following
262 conditions were applied: initial denaturation at 94°C for 3 min, followed by 25 cycles of 94°C
263 for 45 s, 50°C for 45 s and 72°C for 1 min, and a final extension step at 72°C for 5 min. For
264 the ITS gene, the first PCR was carried out under the following conditions: initial
265 denaturation at 95°C for 3min, followed by 25 cycles of 95°C for 30 s, 54°C for 30 s and
266 72°C for 30 s, and a final extension step at 72°C for 5 min. For both genes, the second PCR
267 was conducted with the Nextera XT index kit (Illumina, San Diego, CA) following
268 manufacturer's instructions. Amplicons were verified on 1% agarose gel and purified using
269 AMPure XP magnetic beads (Beckman Coulter, USA). Concentrated samples and negative
270 controls were pooled in equimolar ratios, diluted to 4 nM and sequenced with a 2 x 250 run on
271 an Illumina MiSeq platform at Teagasc Next Generation DNA sequencing facility (Ireland).
272 Sequencing data was deposited in NCBI under the BioProject accession number
273 PRJNA788893.

274 Raw DNA sequencing data were processed using DADA2 (Callahan et al., 2016) in R
275 (version 4.0.2; (R Core Team, 2020). Briefly, for 16S sequences, reads containing ambiguous
276 sequences were removed and based on quality scores, forward and reverse reads were
277 trimmed at 200 and 270 bp, respectively. After denoising using "dada()" function, reads were
278 merged with a minimum overlap of 20 bp and merged reads ranging between 368 and 379 bp

279 were further filtered to remove chimeras using “removeBimeraDenovo()” function. To assign
280 taxonomy, we referred to SILVA SSU r138 database with “IdTaxa()” in the DECIPHER
281 package (Wright, 2016). Likely contaminants were removed with “isContaminant()” in
282 “decontam” package (Davis et al., 2018) using the prevalence method and the negative
283 control. We further removed Amplicon Sequence Variants (ASV) that were not assigned to
284 the domain level and singletons. For the ITS sequences, the sequences were similarly
285 processed, except that for the initial filtering process reads were not trimmed at a defined
286 length (to account for biological variation). After denoising, merged reads with a range
287 between 230 and 413 bp were further filtered to remove chimeras. Taxonomy was assigned
288 using the UNITE v2020 database.

289

290 ***Quantitative PCR amplification (qPCR)***

291 The abundance of microbial communities involved in denitrification and nitrification
292 pathways was estimated by quantitative PCR (qPCR) using SYBR green detection chemistry
293 in a CFX384 touch real-time PCR detection system (BioRad, USA). Marker genes for
294 nitrifiers (*amoA* in archaea, bacteria and comammox), denitrifiers (*nirK* and *nirS*) and N₂O-
295 reducers (*nosZ* clade I and *nosZ* clade II) were quantified as described previously (Fowler et
296 al., 2018; Hallin et al., 2009; Henry et al., 2006; Hornek et al., 2005; Jones et al., 2013;
297 Rotthauwe et al., 1997; Throbäck et al., 2004; Tourna et al., 2008 and details are in Table S2
298 and S3). Additionally, abundance of the microbial community was quantified using
299 crenarchaeota, bacterial and fungal specific genes (Table S3). Standards were prepared from a
300 known concentration of a linearized plasmid containing PCR products of the target genes
301 (Duff et al., 2017), which were serially diluted in Tris-EDTA buffer solution for molecular
302 biology (Invitrogen, Thermo Fisher, USA) to create a standard curve between 10⁷ – 10¹
303 copies / μL. All qPCR assays were performed in duplicate for each sample and standards.
304 When possible, DNA of known microorganisms possessing the gene of interest, was included
305 as a positive control. to remove any inhibitory effects on the amplification, BSA was added to
306 each qPCR reaction mixture (Table S2). Reagents, positive controls used and reaction
307 conditions for each gene are shown in Table S2 and S3. CFX Manager Software was used to
308 calculate gene copy numbers prior to statistical analyses.

309

310 **Statistical analyses**

311 All analyses were performed on R (version 4.0.3). For all statistical analyses involving soil
312 physico-chemical properties and gene abundance, we examined the distribution of the data
313 and square root- or log-transformation was applied when appropriate. Pearson tests were used
314 to evaluate the correlation between each predictive variable and either soil potential
315 nitrification, denitrification, $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio, NH_4 or NO_3 . In all analyses, gene
316 abundances were assessed as copies per ng of DNA, and similar trends were obtained when
317 assessed as a proportion of the total 16S community (data not shown). A Principal Component
318 Analysis was performed to further explore relationships between soil physico-chemical
319 properties, functional genes abundance and the potential denitrification, $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$
320 ratio, NH_4 and NO_3 .

321 For community analyses, normalisation of sequence reads across all samples was not
322 performed because rarefaction curves (using “rarecurve” function in “vegan”; Oksanen et al.,
323 2018) showed that sequence effort was sufficient to reach saturation in all *16S rRNA* (Fig.
324 S1a, 5,453 to 134,011 reads / sample) and ITS (Fig. S1b, 9,625 to 118,915 reads / sample)
325 samples. A soil sample collected in CR containing 2810 ITS reads was excluded from fungal
326 community analyses. To visualize the dissimilarity between either prokaryotic or fungal
327 communities, we used Principal Coordinate Analysis (PCoA), obtained with “wcmdscale()”
328 in “vegan” based on Bray-Curtis distances.

329 To identify which soil properties were best predicting potential activities, we tested soil
330 variables hypothesised to be important in determining N_2O emissions. We created an
331 individual linear mixed-effect model for each potential activity including “Site” as a random
332 effect to account for the sampling design using “lmer()” (“lme4; Bates et al., 2015) and we
333 visually inspected residuals for normality and homoscedasticity. TOC was excluded from all
334 models because it was highly collinear with TN. Using a multimodel inference and model
335 averaging approach with “dredge()” in “MuMIn” (Barton, 2016), we generated several
336 models containing random subsets of the soil variables, followed by “importance()” and
337 “model.avg()” to obtain the relative importance and the confidence interval of each individual
338 parameter. We refitted the model with only the significant variables to obtain the marginal R^2
339 using “r.squaredGLMM()”. In addition, to evaluate the effect of pH on the ratio $\text{N}_2\text{O} / (\text{N}_2\text{O} +$
340 $\text{N}_2)$, we fitted a beta-regression to account for the data ranging between zero and one.

341 We estimated the bacterial or fungal richness and diversity for each sample using the Chao
342 index with “estimateR()” and Shannon index using “diversity()” in “vegan”. To identify
343 significant soil physico-chemical properties that explain variation in the microbial and fungal

344 communities, we performed a stepwise model selection using permutation tests with
345 “ordistep” (“vegan”) and then assessed the amount of variation explained by these properties
346 with distance-based redundancy analyses (db-RDA) using “capscale”.

347 To evaluate the effect of the bacterial or fungal community composition on either potential
348 activity or $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio, we fitted individual general additive model using the first
349 two axes of the PCoA as predictors of each potential activity using “ordisurf()” from “vegan”.
350 This method takes non-linear relationships into account, provides an estimation of the
351 variation in the response variable that is explained by the community structure and
352 implements the fitted values on the ordination. In addition, we evaluated whether the relative
353 abundance of the microbial and fungal phylum was correlated with either potential activity or
354 $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio using Pearson correlation tests.

355 To quantify the amount of variation explained by soil physico-chemical properties, functional
356 gene abundance and overall microbial community composition we performed individual
357 variation partitioning using “varpart()” function in “vegan” for each potential activity. For this
358 test, the contribution of the microbial community composition was evaluated by extracting the
359 first two axes of the PCoA. Variables were standardised prior variation partitioning.

360

361 **Results**

362 **Climatic conditions and soil physico-chemical properties**

363 Mean annual temperature across all sites averages 9.45°C , varying between 7.5°C and 10.3°C
364 and mean annual precipitation averages 1053 mm, ranging between 832 mm and 1249 mm
365 (Table S1). All measured soil physico-chemical properties for each site are in Table S4.
366 Briefly, regarding the soil texture, sand content varied between $11.4 \pm 1.4 \%$ and 59.4 ± 2.6
367 $\%$, silt between $24.2 \pm 0.2 \%$ and $55.1 \pm 1.1 \%$ and clay between $11.4 \pm 1.4 \%$ and $37.2 \pm$
368 5.7% . Bulk density ranged between $0.6 \pm 0.1 \text{ g / cm}^3$ and $1.3 \pm 0.04 \text{ g / cm}^3$. The range of
369 pH varied between 4.8 and 7.7. Soil chemical properties such as P varied greatly within sites
370 due to the differing fertiliser applications and land management *in situ*. Across all samples, P
371 varied between 10 and 286 mg / L and total N and C varied between 0.25 to 1.25 % and 2.34
372 to 14.5 %, respectively. Potassium and Magnesium varied between 20.0 and 760.0 mg / L and
373 57 and 375 mg / L, respectively.

374

375 **Soil potential nitrification, denitrification and N₂O emissions**

376 Soil potential nitrification averaged 18.6 mg N kg⁻¹ dry soil day⁻¹ across all soil samples,
377 ranging from 1.9 ± 1.3 mg N kg⁻¹ dry soil day⁻¹ in CR and 50.2 ± 10.4 mg N kg⁻¹ dry soil day⁻¹
378 ¹ in SC (Fig. 2a). Soil potential denitrification averaged 22.3 mg N₂O-N kg⁻¹ dry soil day⁻¹
379 across all soil samples. A wide difference of potential total denitrification rates was found
380 between sites, ranging from 7.1 ± 0.8 and 7.1 ± 1.3 mg N₂O-N kg⁻¹ dry soil day⁻¹ in LY and
381 CG, respectively, up to 48.6 ± 16.8 and 42.0 ± 18 mg N₂O-N kg⁻¹ dry soil day⁻¹ at OP and AT
382 respectively (Fig. 2b). Although the N₂O / (N₂O + N₂) ratios varied between 0.65 ± 0.1 in OP
383 and 0.96 ± 0.04 in TI (Fig. 2c), 75% of the ratios were above 0.71 indicating that most N
384 potential emissions were in the form of N₂O rather than N₂ under the conditions tested. (Fig.
385 2c).

386

387 **Abundance of total and functional microbial communities**

388 Considering the overall microbial communities, total bacteria were the most abundant,
389 ranging from 7.6 × 10⁴ and 4.7 × 10⁵ gene copy number ng⁻¹ DNA (Table S5). Fungal
390 abundance ranged between 1.9 × 10³ to 1.1 × 10⁴ and crenarchaeota abundance ranged between
391 7.4 × 10² and 1.2 × 10⁴ gene copy number ng⁻¹ DNA (Table S5). The most abundant microbial
392 nitrifiers were the AOA, ranging from 1.7 × 10³ to 1.6 × 10⁴ gene copy number ng⁻¹ DNA. The
393 abundance of AOB ranged between 8.6 × 10² and 9.7 × 10³ gene copy number ng⁻¹ DNA, while
394 comammox bacteria ranged between 2.2 × 10² and 1.6 × 10³ gene copy number ng⁻¹ DNA.
395 Regarding denitrifying microbes, we observed that nitrite reducer abundance with the Cu-
396 containing enzyme encoded by *nirK* gene was higher than the abundance of those with
397 cytochrome *cd1* nitrite reductase encoded by *nirS* gene, with *nirK* abundance ranging from
398 1.8 × 10³ to 1.1 × 10⁴ and *nirS* from 1.4 × 10² and 1.1 × 10³ gene copy number ng⁻¹ DNA. N₂O
399 reducer abundance varied between 8.2 × 10² and 4.5 × 10³ for *nosZI* and between 6.0 × 10³ and
400 7.9 × 10⁴ gene copy number ng⁻¹ DNA for *nosZII*.

401

402 **Soil microbial community composition**

403 We obtained 14,394,872 prokaryotic and 16,194,189 fungal forward reads associated with
404 reverse reads, and after quality control 7,136,044 prokaryotic and 6,398,066 fungal merged
405 reads remained for analyses. Overall, 91.6 % of the bacterial and archaeal ASVs were

406 identified to the phylum level. Among the 44 identified phyla, Proteobacteria (28 %),
407 Actinobacteriota (15 %), Acidobacteriota (14 %), Verrucomicrobiota (10 %), Bacteroidota (8
408 %) and Planctomycetota (6.7 %) were the most abundant overall and across all sites (Fig. S2).
409 Other less abundant phyla present in all soil samples were observed: Myxococcota (4%),
410 Nitrospirota (1.7 %), Cyanobacteria (1.4 %), Crenarchaeota (1.4 %) Firmicutes (1 %) and
411 Bdellovibrionota (0.2 %) (Fig. S2). For the fungal community, 98 % of the ASVs were
412 identified to the phylum level. A total of 15 phyla were observed, and the most abundant were
413 Mortierellomycota (41.6 %), Ascomycota (37.9 %), Basidiomycota (13.4 %), Glomeromycota
414 (5.6 %) and Chytridiomycota (1 %) (Fig. S2).

415

416 **Abiotic factors associated with potential nitrification, denitrification and N₂O emissions**

417 Soil potential denitrification was highly correlated with soil potential nitrification (Table S6),
418 and similar soil properties were associated with both processes. The best predictors for soil
419 potential nitrification were pH, TN and bulk density with the same importance, followed by
420 K, P and Mg (Table 1) and, when evaluating the best fitting model, all variables were
421 positively correlated with potential nitrification ($R^2 = 0.55$). For potential denitrification, the
422 best fitted model ($R^2 = 0.62$) included pH, TN and P, all presenting the same weights
423 (importance) to the model prediction and positively correlated to potential denitrification
424 (Table 1). Soil pH was the only predictor for N₂O / (N₂O + N₂) ratio (Table 1, $R^2 = 0.28$). To
425 quantify this relationship, we refitted a beta-regression including only soil pH and we
426 observed a negative relationship between pH and N₂O / (N₂O + N₂) ratio, meaning that the
427 increase in soil pH favours the decrease of N₂O emissions in relation to total emissions (N₂O
428 + N₂) (beta-regression; z-value = -6.6, pseudo- $R^2 = 0.1$, p-value < 0.01; Fig. S3).

429 Neither the mean annual precipitation nor the mean annual temperature were correlated with
430 the potential activities nor N₂O / (N₂O + N₂) ratio (Table S6).

431 **Biotic factors associated with potential nitrification, denitrification and N₂O emissions**

432 None of the soil physico-chemical properties were strongly associated with the functional
433 gene abundances (Fig. S4), and in return these were weakly associated with the potential
434 activities. The nitrifiers abundance AOA, AOB and comammox were positively correlated
435 with potential nitrification ($r < 0.4$; Table S6). For the denitrifiers, only *nirS* and *nosZ* clade I
436 abundance were positively correlated with the potential denitrification (Table S6). No

437 evidence was found of an association between gene abundances and potential $\text{N}_2\text{O} / (\text{N}_2\text{O} +$
438 $\text{N}_2)$ ratio (Table S6).

439 We then evaluated the influence of the microbial and fungal community richness, diversity
440 and composition on the potential denitrification and on the $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ end-product.
441 The microbial community richness and diversity were positively correlated with the potential
442 denitrification, but such relationships were weak (Coefficient $r < 0.3$; Table S6,) and only the
443 microbial diversity was significantly associated with the $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio (Table S6).
444 For the fungal community, only the Shannon diversity index was weakly and negatively
445 correlated to the potential nitrification (Coefficient $r < -0.3$; Table S6). Soil physico-chemical
446 properties including pH, P, TN, Mg, K, bulk density and clay were significantly correlated
447 with soil microbial community's composition (ordistep; p-values < 0.01) and explained 20 %
448 and 14 % of the variation in the microbial and fungal communities, respectively (Fig. S5).

449 Both the prokaryotic and fungal community structure influenced both potential activities and
450 the $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio (Fig. 3 and Table 2). The predicted values (splines) follow the
451 same trends as the observed values (symbols; Figure 3), suggesting that the community
452 composition is a good predictor of both potential activities and the $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio.
453 Such trends are confirmed based on the generalised additive models (Table 2). The deviance
454 explained by the prokaryotic community composition was higher than the fungal community
455 (Table 2). In addition, the fungal community composition only explained 6.7 % of the $\text{N}_2\text{O} /$
456 $(\text{N}_2\text{O} + \text{N}_2)$ ratio while the prokaryotic community composition explained up to 23.8 %
457 (Table 2). We also observed that overall, the communities with higher nitrification levels (Fig.
458 3a, d) were also communities with higher denitrification levels (Fig. 3b, e) but lower $\text{N}_2\text{O} /$
459 $(\text{N}_2\text{O} + \text{N}_2)$ ratio (Fig. 3c, f), suggesting that communities with higher potential nitrification
460 and denitrification release less N_2O as a proportion of total emissions.

461 We found some significant relationships between the relative abundance of specific phyla and
462 both potential activities and the $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio but all were moderate (below 0.4).
463 Actinobacteriota, Chloroflexi and Crenarchaeota were positively associated with potential
464 nitrification and denitrification (Fig. S6, Fig. S7). Actinobacteriota and Crenarchaeota were
465 both negatively associated with the $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio (Fig. S8), suggesting that an
466 increase in the abundance of these phyla correlates to an increase in N-transformation rates
467 but a decrease in the $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio. The opposite trend was observed with
468 Verrucomicrobiota and Myxococcota, where an increase in their abundance were associated
469 with a decrease of potential activities and an increase in $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio (Fig S6, Fig.

470 S7, Fig. S8). Regarding the fungal communities, Mortierellomycota and Ascomycota were
471 positively and negatively associated with both potential activities, respectively (Fig. S19,
472 S10). None of the fungal phyla were correlated to the $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio (Fig. S11).

473

474 **Relative contribution of biotic and abiotic factors to soil potential nitrification,** 475 **denitrification and N_2O emissions**

476 We evaluated the relative contribution of soil physico-chemical properties, functional genes
477 abundance and prokaryotic community composition to the potential activities and $\text{N}_2\text{O} / (\text{N}_2\text{O}$
478 $+ \text{N}_2)$ ratio. The potential of nitrification and denitrification were mainly explained by the
479 physico-chemical properties of the soil (32 % and 29 %, respectively) (Fig. 4). An additional
480 20 and 16 % of the variability was explained by the community composition together with the
481 soil physico-chemical properties. On their own the soil functional genes explained between 0
482 and 3 % of the variability of both potential activities. In addition, soil properties together with
483 community structure explained up to 18 % of the variation in the $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ ratio,
484 while soil properties alone explained 4 % (Fig. 4).

485

486 **Discussion**

487 Reduction of N_2O emissions remains among the global challenges to mitigate climate change.
488 It is therefore crucial to identify the key factors influencing the potential of agricultural soils
489 to release N in the form of N_2O . However, our ability to predict N_2O emissions at large scales
490 remains limited due to the multitude of factors and players involved in the processes leading
491 to the release of N-gases. In this study, we aimed to identify the main factors driving potential
492 N_2O emissions on a national scale, and the relative importance of the biotic and the abiotic
493 factors in this regard. Thirty-two contrasting sites across Ireland resulted in large differences
494 of potential denitrification in managed grasslands soils (up to $41.5 \text{ mg } \text{N}_2\text{O-N kg}^{-1} \text{ soil day}^{-1}$).
495 We found that soils with higher potential to nitrify were also soils that had the highest
496 potential to perform denitrification, and similar factors were associated with both processes
497 rates. In addition, most of the potential N denitrified was emitted in the form of N_2O rather
498 than N_2 under the conditions tested, as observed in previous studies (e.g., Laughlin & Stevens,
499 2002). Nitrification is an important regulator of soil nitrogen availability for plants as N
500 availability increases by converting the less mobile NH_4^+ into a more mobile form NO_3^- , and

501 conversely denitrification results in N gaseous loss from the soil. Consequently, parameters
502 that were associated with high potential nitrification and denitrification may drive a higher N
503 turnover in soils and result in higher emissions from Irish grasslands. While nitrification may
504 also contribute to N₂O emissions (Hooper et al., 1997; Kozłowski et al., 2016), potential N₂O
505 emissions were only measured in conditions favouring denitrification in our study as it is the
506 predominant N₂O production process in Irish grasslands (Krol et al., 2016).

507 The potential denitrification was regulated by soil pH, TN and P, but only soil pH influenced
508 the denitrification end-product N₂O / (N₂O + N₂) (Table 1). The important role of soil pH on
509 N₂O emissions has been demonstrated for decades and in numerous studies, either by
510 impeding the assemblage of the N₂O reductase enzyme or via influencing the soil microbial
511 community composition (e.g., Firestone et al., 1980; Samad et al., 2016; Šimek & Cooper,
512 2002; Liu et al. 2010). Not surprisingly, soil TN was also a relevant predictor of the soil
513 potential denitrification, as it is a substrate for nitrification and denitrification processes, and
514 the concentration of N species in soil influenced N released as N₂O gas (Table S6).
515 Interestingly, soil P content was as important as TN and pH in predicting denitrification rates.
516 Our results show that P may be a key soil property to take into account to predict more
517 accurately potential denitrification in large-scale field studies. Others have reported the
518 potential role of P in denitrification within experimental trials with contrasting results, with
519 either P-addition favouring (Mehnaz et al., 2019; Mehnaz & Dijkstra, 2016) or reducing N₂O
520 emissions (O'Neill et al., 2020). Soil P has also observed to be positively correlated with total
521 nitrification, mineralisation and immobilisation (O'Neill et al., 2021). In our study, we found
522 higher potential denitrification from soils with higher P-levels. The way by which P
523 stimulates denitrification remains unclear, but an overall P-effect on the N-cycle may be due
524 to reducing soil nutrient limitation and thus, influencing the microbial activity (Mehnaz et al.,
525 2019; Mehnaz & Dijkstra, 2016) and/or community structure (Randall et al., 2019). Although
526 no relationship between P and the functional gene abundance was found in our study, such
527 effects remain to be tested using experimental approaches that may provide mechanistic
528 insights into the impact of P on soil microbial communities and resulting N₂O emissions. In
529 addition, others have found that the effects of P on N₂O emissions were dependent on C-
530 substrate availability (O'Neill et al., 2020), but this could not be evaluated in our study as soil
531 carbon content was highly collinear with nitrogen at our sites.

532 Soil biological properties also contributed to drive the soil potential denitrification and the
533 end-product N₂O / (N₂O + N₂) in Irish grasslands. The prokaryotic community was a better

534 predictor of the potential denitrification than the fungal communities (33.5 % vs 19.5 %), and
535 the level of prediction of the fungal community composition on the end-product $\text{N}_2\text{O} / (\text{N}_2\text{O} +$
536 $\text{N}_2)$ was weak compared to prokaryotic communities (6.7 % vs 23.8 %). As fungi lack the
537 N_2O reductase converting N_2O into N_2 , it was therefore expected that this community would
538 influence the end-product (Maeda et al., 2015). However, our findings are in accordance with
539 experimental studies using bacterial or fungal inhibitors showing that bacteria are more
540 important contributors to denitrification (Maeda et al., 2017), although others have found that
541 the relative role of bacteria and fungi may depend on soil conditions such as pH and soil
542 moisture (Chen et al., 2015). It is interesting to note that several studies have shown the
543 influence of the fungal phylum Glomeromycota, known for their association with plant roots
544 through the mycorrhization process, on N_2O emissions (Gui et al., 2021; Storer et al., 2018;
545 Wagg et al., 2014). However, we found no evidence supporting the relationship between the
546 relative abundance of this fungal community and the potential N_2O emissions released from
547 Irish soils.

548 A noteworthy observation was that the soil prokaryotic communities associated with higher
549 potential nitrification and higher potential denitrification were more likely to favour complete
550 denitrification up to N_2 (Fig. 3). These observations remain correlational in nature as the
551 relationship between communities and N_2O emissions may be in part explained by a
552 confounding effect of the soil physico-chemical properties. However, we found that the
553 presence and relative abundance of certain prokaryotic phyla showed the same trend, with the
554 increasing abundance of Crenarchaeota and Actinobacteriota also associated with higher
555 nitrification, denitrification and a lower $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$. Interestingly, the capacity to
556 nitrify and denitrify is present in members of the Crenarchaeota but Actinobacteriota are not
557 able to perform complete denitrification as *nosZ* genes producing N_2O reductase are absent
558 from their genomes (Hallin et al., 2018). Therefore, the association between Actinobacteriota
559 and $\text{N}_2\text{O} / (\text{N}_2\text{O} + \text{N}_2)$ remains unclear but our results suggest a role of this bacterial
560 community in overall decreasing N_2O emission relative to N-gases.

561 The soil microbial community structure, including diversity and composition, were better
562 predictors of the potential denitrification and of the end-product ratio than the functional
563 genes abundance. Only *nirS* and *nosZ* clade I were related to denitrification potential but
564 when evaluated together with community structure and soil physico-chemical properties, their
565 contribution explaining soil denitrification remained below 3%. Interestingly, Domeignoz-

566 Horta et al., (2018) showed that soil properties mainly explained N₂O emissions at low rates
567 while peaks of emissions were mostly explained by denitrifier diversity and abundance. In our
568 study, the lack of variability explained by functional gene abundances relative to other factors
569 is likely due to several aspects. First, denitrification is a facultative process and potential
570 denitrification is related to the size of the enzyme pool at the time of sampling while
571 functional gene abundances reflect the number of microorganisms genetically capable to
572 denitrify. Second, the moderate relationship may also be attributed to denitrification being a
573 modular process with numerous steps, making prediction difficult with each functional gene
574 being involved in one step. For example, *nirK* abundance is more correlated to activity in low
575 pH soils (Bowen et al., 2020) and the great majority of our soil had a pH greater than 5.5,
576 possibly not favouring the expression of this functional gene precursor of the denitrification
577 process. Using transcriptional analysis (mRNA) of functional genes may be more
578 representative of the activity of organisms driving reactions in the N-cycle, and therefore may
579 show a stronger correlation with potential N₂O emissions. However, even with gene
580 transcripts, that is not always the case (Frostegård et al., 2021) and some of the process
581 control (e.g. with *NosZ*) are known to be post-transcriptional. Third, it has been suggested that
582 soil microbial community composition and diversity, including that of functional
583 communities, may be better indicators of the soil potential denitrification by providing
584 additional information that is not accounted for with the functional gene abundance alone,
585 such as interactions between species or niche complementarity (Graham et al., 2016). Further
586 experimental investigation is needed to elucidate the interaction between species interactions
587 and / or niche complementarity and N₂O emissions under controlled conditions. Overall, our
588 results highlight that at larger scales, prokaryotic community structure rather than
589 denitrification gene abundance, may improve prediction of denitrification rates.

590 Other large-scale studies in New Zealand (Morales et al., 2015) and in China (Kou et al.,
591 2019) assessed potential denitrification using soil incubations. They showed similar effects of
592 the soil physico-chemical properties and soil microbial communities, but also highlighted the
593 determinant role of latitude and climate on the microbial communities and their N₂O
594 emissions. Using similar methods, we found no evidence supporting that climate (Table S6)
595 nor latitude (data not shown) were related to soil potential denitrification. While N₂O
596 measurements are known to be strongly impacted by environmental and climatic conditions *in*
597 *situ* (e.g., Kiese & Butterbach-Bahl, 2002; Rafique et al., 2011), the lack of an effect observed
598 here may be partly attributed to the fact that potential assays are performed under optimal

599 conditions and therefore not accounting for factors regulating the activity of microorganisms
600 and of the synthesized enzymes. As such it is important to note that potential assays provide
601 mechanistic insights but do not infer actual rates *in situ* (Hazard et al., 2020). However, our
602 findings may also highlight the fact that the relative importance of predictors is scale-
603 dependent but may also vary across regions depending on limiting factors. For example, Kou
604 et al. (2019) that found that mean annual precipitation was a key factor in arid and semi-arid
605 environments but not in mesic sites. In addition, Ireland is characterised by a relatively small
606 total land area and low variability across its territory in terms of climatic conditions, which
607 may also explain the lack of influence on the resulting potential denitrification and N₂O
608 emissions.

609 In conclusion, we showed that a few key determinants may be considered to improve
610 predictions of N₂O emissions from managed grasslands at large scales, namely soil pH, TN,
611 P, and prokaryotic community structure. While the soil physico-chemical properties were
612 most predictive of N₂O emissions, the addition of prokaryotic community structure data
613 enhanced the level of predictions. We identified certain phyla that were associated with higher
614 denitrification and a lower N₂O / (N₂O + N₂). Soils with higher potential to nitrify were also
615 soils that had the highest potential to perform denitrification, and similar factors were
616 associated with both processes rates. Therefore, considering such factors is important to
617 predict N-transformations at larger scales, and to manage soils in a manner that will reduce
618 gaseous losses of nitrogen from agricultural grasslands.

619

620

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633

634 **Authors' contribution**

635 CD, KR, FB, PRP designed the study. CD, AD, PRP and JR collected and processed samples.
636 CD performed the bioinformatics, statistical analyses, and interpreted results. CD wrote the
637 manuscript with help from FB, PRP and MV, and all authors revised this manuscript.

638

639 **Data accessibility**

640 All data will be archived in Figshare from July 2022, except information regarding farms
641 location and management, at the following link:
642 <https://doi.org/10.6084/m9.figshare.19337105.v2>. Scripts with the data will be uploaded on
643 bitbucket (https://bitbucket.org/Coline_Dev/IrishDenitrification-MINE/). Raw DNA
644 sequencing data are available on the NCBI database under the accession number BioProject
645 PRJNA788893.

646

647 **Tables**

648 Table 1. Relative importance of soil physicochemical variables for predicting variation in
649 potential nitrification, denitrification and the $N_2O / (N_2O + N_2)$ end-product. Table includes
650 the relative variable importance (corresponds to the sum of “Akaike weights” across models
651 including each variable) and the model-averaged confidence interval for each estimated
652 parameter.

653 Table 2. Role of the soil microbial communities in determining the potential nitrification,
654 denitrification and $N_2O / (N_2O + N_2)$ end-product emissions based on generalised additive
655 models.

656 Table S1. Description of the sites sampled, including the abbreviations used in this study for
657 each site, their location, mean annual precipitation and temperature, and land management
658 including the type of fertilisers applied, the type of livestock and stocking rates, and whether
659 there is soil drainage on site.

660 Table S2. Reagents and quantities used for qPCR of selected genes.

661 Table S3. Reaction conditions, primer final concentration and positive control for qPCR of
662 selected genes.

663 Table S4. Soil physico-chemical properties at each sampling site. Values are averaged per site
664 with the associated standard error.

665 Table S5. Gene abundance (gene copy number per ng of DNA) at each sampling site. Values
666 are averaged per site with the associated standard error.

667 Table S6. Relationship between each variable and the nitrate, ammonium, potential
668 nitrification, denitrification and $N_2O / (N_2O + N_2)$ ratio. Relationships are based on Pearson's
669 correlations and values show the coefficient r . Bolded values show significant relationships,
670 with ** showing significance at p -values < 0.01 and * showing p -values < 0.05 .

671

672 **Figures**

673 Figure 1. Map of Ireland showing the location of the sampling sites. Detailed information
674 about the sampling sites can be found in Table S1.

675 Figure 2. Potential nitrification (a), denitrification (b) and $N_2O / (N_2O + N_2)$ ratio (c)
676 according to the sampling sites. Bar graph shows the average and error bars represent standard
677 errors. Site descriptions are in Table S1. Numbers of replicates per site are $n = 3$ for BA, BG,
678 BE, BK, CA, GL, HS, HI, KK, CF, ST, SC; $n = 4$ for CR, LY, DU, GR, MO, RO, SO; $n = 5$
679 for AH, AT, BL, CD, CL, CG, DO, HN KI, OP, PA, TI, $n = 12$ for JC.

680 Figure 3. Principal Coordinate Analysis (PCoA) ordinations showing the prokaryotic (left)
681 and fungal communities (right) and associated potential nitrification (a, d), denitrification (b,
682 e) and $N_2O / (N_2O + N_2)$ ratio (c, f) measured from each soil sample. Each dot represents a
683 community from a soil sample and the colour of the dot shows the level of nitrification (a,d)
684 or denitrification rates (b,e) measured in the soil sample from which the community was
685 characterised (colour varies from blue (lower rates) to red (higher rates) and for potential
686 nitrification rates are expressed as $mg\ N / kg\ day$ and for potential denitrification rates as mg
687 $N_2O-N / kg\ day$). For the bottom panels, the size of the dots shows the $N_2O / (N_2O + N_2)$ ratio
688 associated with the community. Splines show the expected rates associated with the
689 communities' composition based generalised additive models. Linear relationships are

690 observed when the splines are parallel and non-linear relationships are observed with curved-
691 lines.

692 Figure 4. Relative contribution of soil physico-chemical properties, gene abundance and
693 microbial community structure on the potential nitrification (a), denitrification (b) and $N_2O /$
694 $(N_2O + N_2)$ ratio (c), based on variation partitioning. Microbial community structure was
695 obtained by extracting the first two axes of the Principal Coordinate Analysis (PCoA).
696 Residuals show the amount of variance that remained unexplained.

697 Fig. S1. Rarefaction curves of the prokaryotic (a) and fungal (b) communities for each
698 sample.

699 Fig. S2. Distribution of prokaryotic (a) and fungal (b) taxa at the different sampling sites. The
700 relative abundance was based on read numbers.

701 Fig. S3. Relationship between pH and the potential denitrification (a) and $N_2O / (N_2O + N_2)$
702 ratio (b). Lines show the linear (a) and beta (b) regression between variables.

703 Fig. S4. Principal component analysis (PCA) ordination of the soil bio physico-chemical
704 properties based on Euclidean distances. Each point represents measurements taken from a
705 soil sample, the colour indicates the denitrification rates measured in from the soil sample
706 (colour varies from blue (lower rates) to red (higher rates), expressed as $mg\ N_2O-N / kg\ day$).
707 The size of the dot shows the $N_2O / (N_2O + N_2)$ ratio associated with the community. The text
708 indicates the loadings associated with each soil property: phosphorus ("P"), ammonium
709 (NH_4) , nitrate (" NO_3 "), potassium ("K"), magnesium ("Mg"), total organic carbon ("TOC"),
710 total nitrogen ("TN"), copper ("Cu"), pH, percentage of sand ("sand"), silt ("silt") and clay
711 ("clay"), bulk density, and gene abundance including crenarchaeota ("Archaea"), fungi,
712 bacteria, complete ammonia-oxidizing bacteria ("COMAMMOX"), ammonia-oxidizing
713 archaea ("AOA"), ammonia-oxidizing bacteria ("AOB"), nitrate reductase genes ("nirS" and
714 "nirK"), nitrous oxide reductase genes ("nosZI" and "nosZII").

715 Fig. S5. Distance-based redundancy analyses (db-RDA) showing soil physico-chemical
716 properties significantly correlated with the prokaryotic (a) and fungal (b) communities. Each
717 point represents a community from a soil sample, and the arrows and text indicate the
718 loadings associated with each soil property: phosphorus ("P"), potassium ("K"), magnesium
719 ("Mg"), total nitrogen ("TN"), pH, percentage of clay ("clay") and bulk density. Significant
720 properties were identified performing a forward model selection using permutation tests.

721 Fig S6. Relationship between the relative abundance of each prokaryotic phyla and the
722 potential nitrification rates. The relative abundance was based on read numbers. The text
723 shows the coefficient r based on Pearson's correlation and the associated p-value.

724 Fig S7. Relationship between the relative abundance of each prokaryotic phyla and the
725 potential denitrification rates. The relative abundance was based on read numbers. The text
726 shows the coefficient r based on Pearson's correlation and the associated p-value.

727 Fig S8. Relationship between the relative abundance of each prokaryotic phyla and the
728 potential $N_2O / (N_2O + N_2)$ ratio. The relative abundance was based on read numbers. The
729 text shows the coefficient r based on Pearson's correlation and the associated p-value.

730 Fig S9. Relationship between the relative abundance of each fungal phyla and the potential
731 nitrification rates. The relative abundance was based on read numbers. The text shows the
732 coefficient r based on Pearson's correlation and the associated p-value.

733 Fig S10. Relationship between the relative abundance of each fungal phyla and the potential
734 denitrification rates. The relative abundance was based on read numbers. The text shows the
735 coefficient r based on Pearson's correlation and the associated p-value.

736 Fig S11. Relationship between the relative abundance of each fungal phyla and the potential
737 $N_2O / (N_2O + N_2)$ ratio. The relative abundance was based on read numbers. The text shows
738 the coefficient r based on Pearson's correlation and the associated p-value.

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742 Table 1. Relative importance of soil physicochemical variables for predicting variation in
743 potential nitrification, denitrification and the $N_2O / (N_2O + N_2)$ ratio. Table includes the
744 relative variable importance (corresponds to the sum of "Akaike weights" across models
745 including each variable) and the model-averaged confidence interval for each estimated
746 parameter.

Model	Variables	Relative variable importance	Confidence interval	
			2.5 %	97.5 %

Potential nitrification	pH	1	0.68	1.34
	TN	1	3.57	7.16
	Bulk density	1	3.69	10.94
	K	0.82	0.13	2.01
	P	0.78	0.09	1.75
	Mg	0.68	0.05	0.24
	clay	0.03	-0.01	0.05
Potential denitrification	pH	1	0.18	0.41
	P	1	0.16	0.27
	TN	1	0.5	0.91
	Bulk density	0.23	-0.41	0.78
	K	0.07	-0.08	0.18
	Mg	0.03	0	0.02
	clay	< 0.01	0	0
ratio N ₂ O / (N ₂ O + N ₂)	pH	1	-0.19	-0.1
	Bulk density	0.33	-0.52	0.22
	K	0.31	-0.14	0.06
	clay	0.3	0	0.01
	TN	0.3	-0.12	0.21
	P	0.29	-0.13	0.07
	Mg	0.26	-0.01	0.01

747

748 Table 2. Role of the soil microbial communities in determining the potential nitrification,
749 denitrification and N₂O / (N₂O + N₂) ratio based on generalised additive models.

Soil microbial community	Activity	Deviance explained (%)	P-value
Prokaryotic	Nitrification	31.6	< 0.01

community	Denitrification	33.5	< 0.01
	N ₂ O / (N ₂ O + N ₂) product	23.8	< 0.01
Fungal community	Nitrification	24.1	< 0.01
	Denitrification	19.3	< 0.01
	N ₂ O / (N ₂ O + N ₂) product	6.7	0.01

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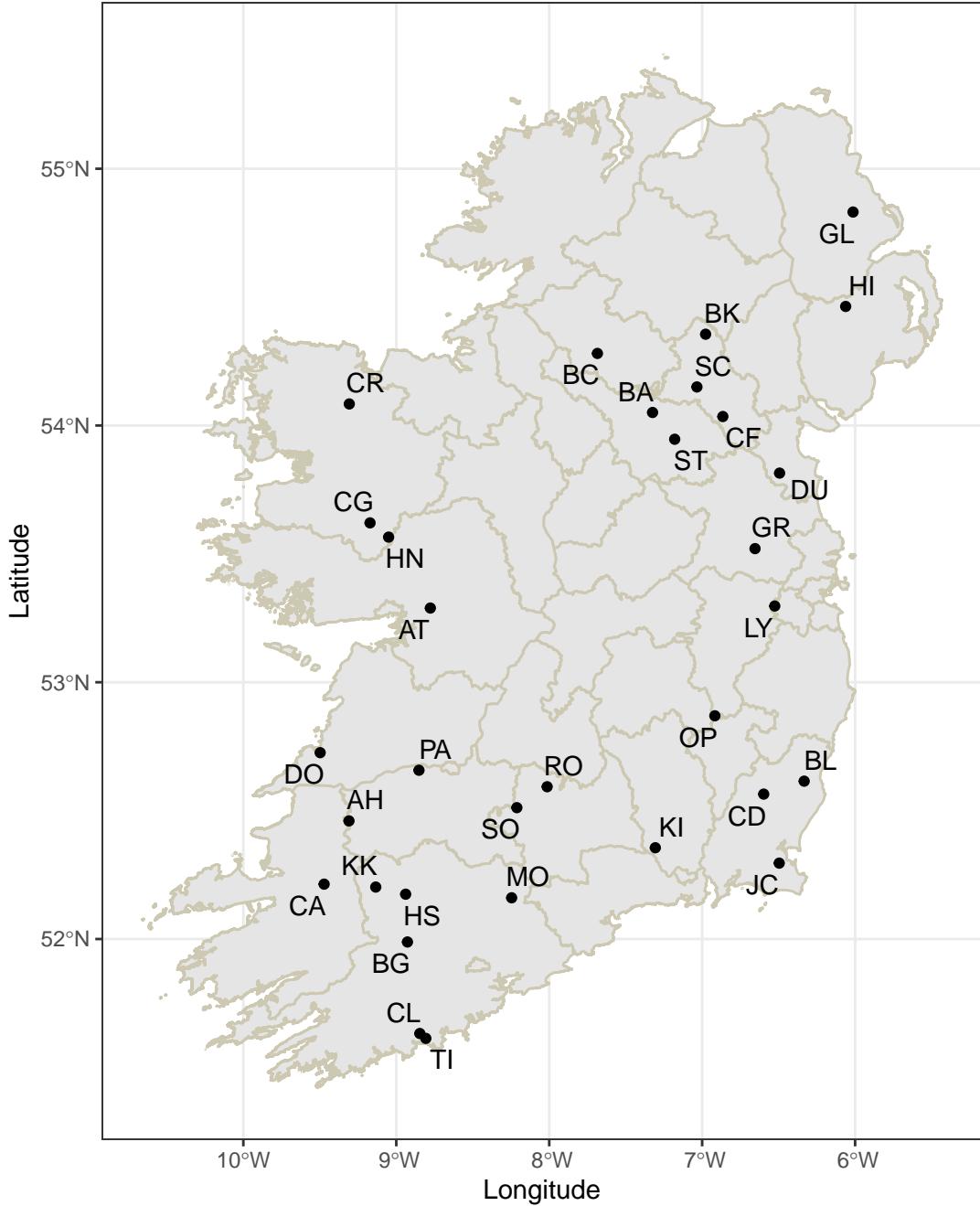
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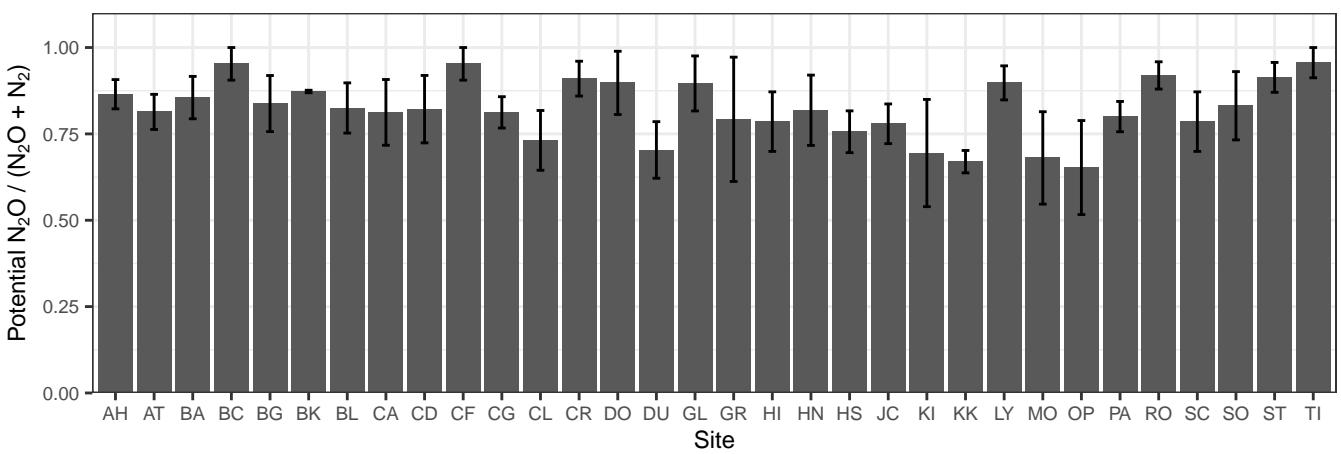
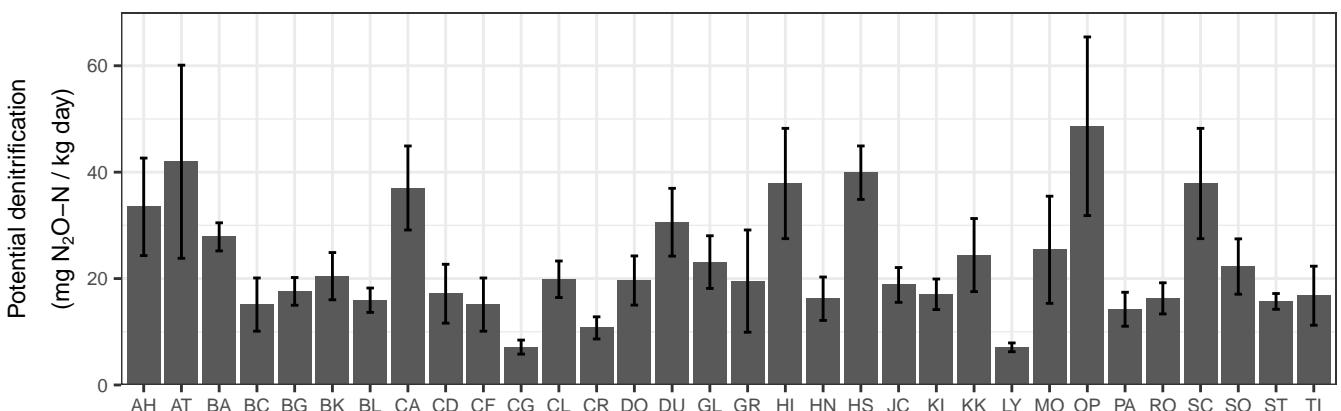
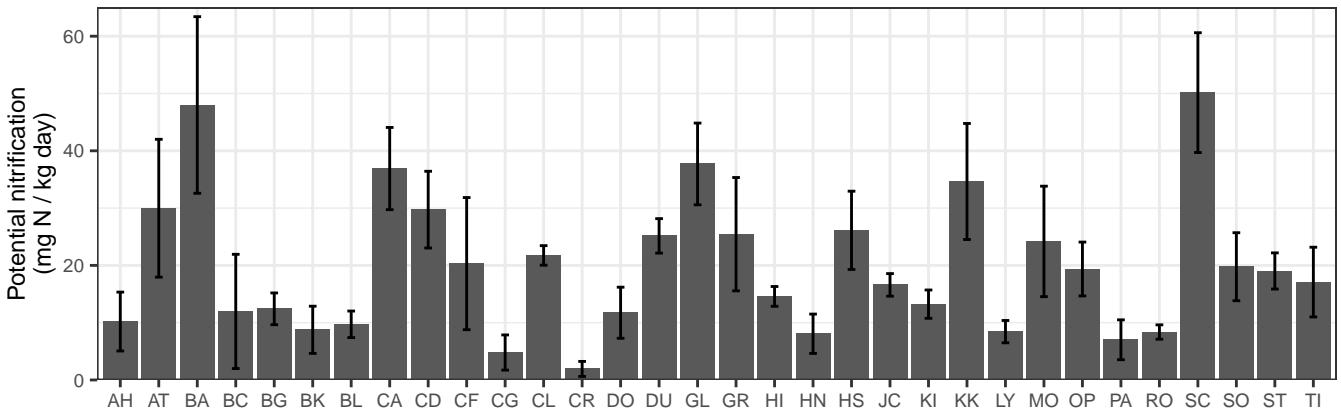
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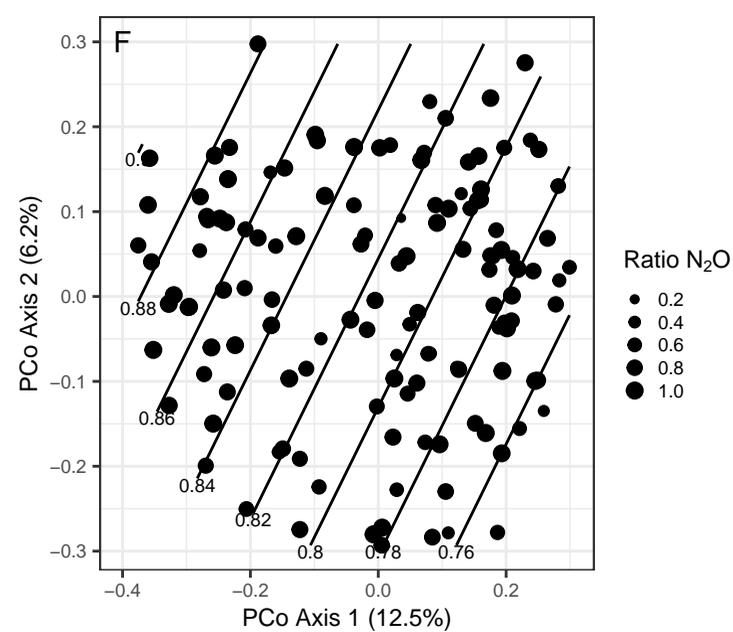
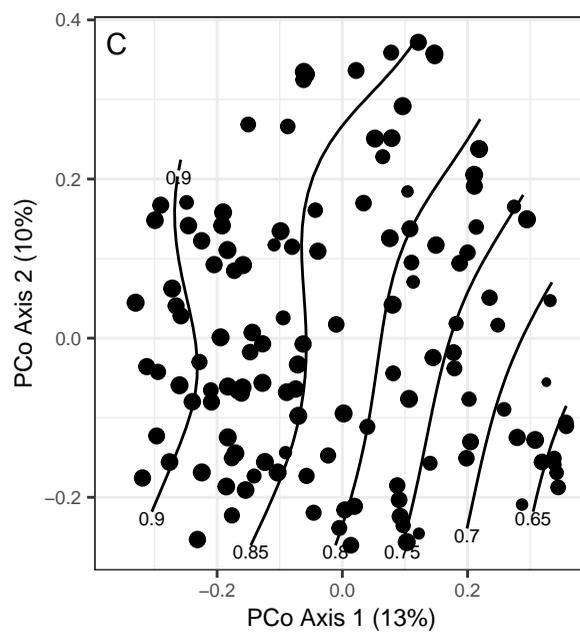
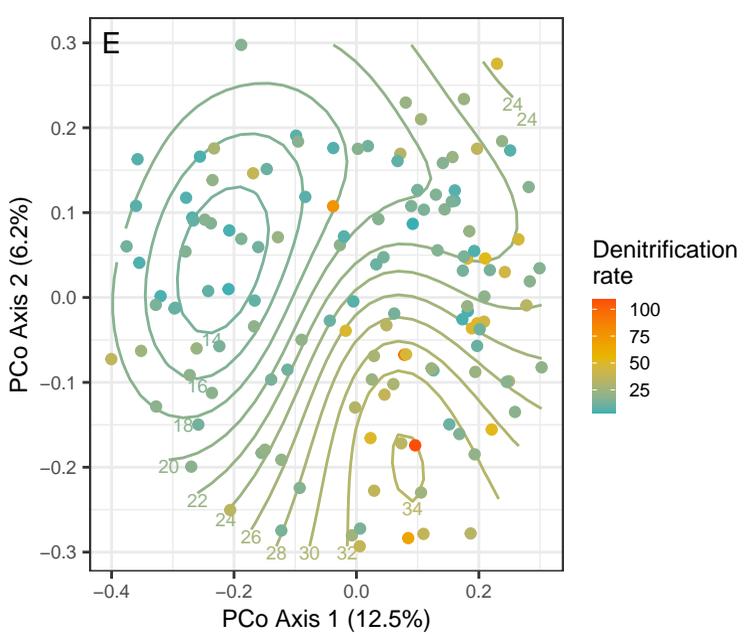
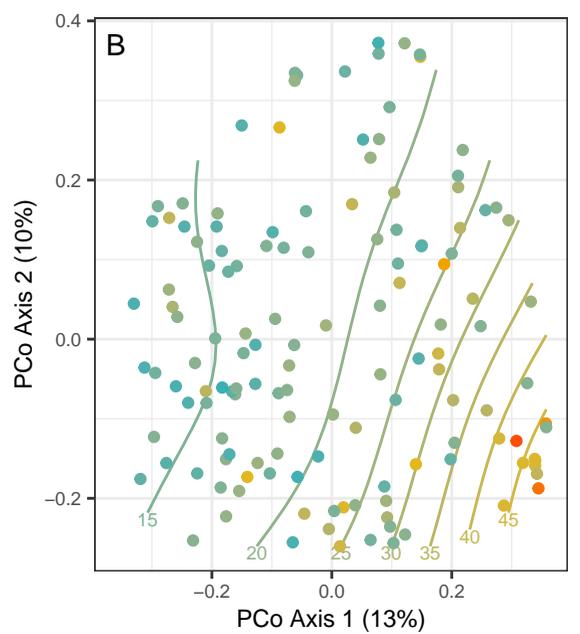
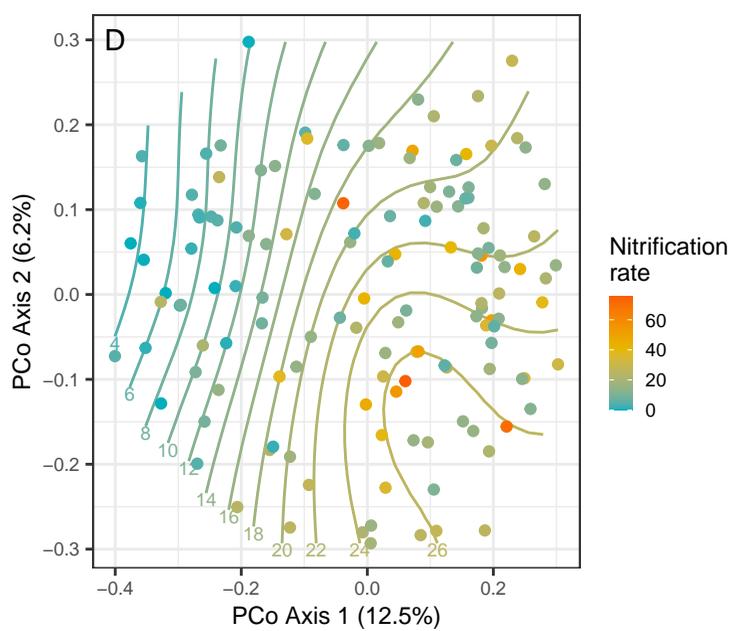
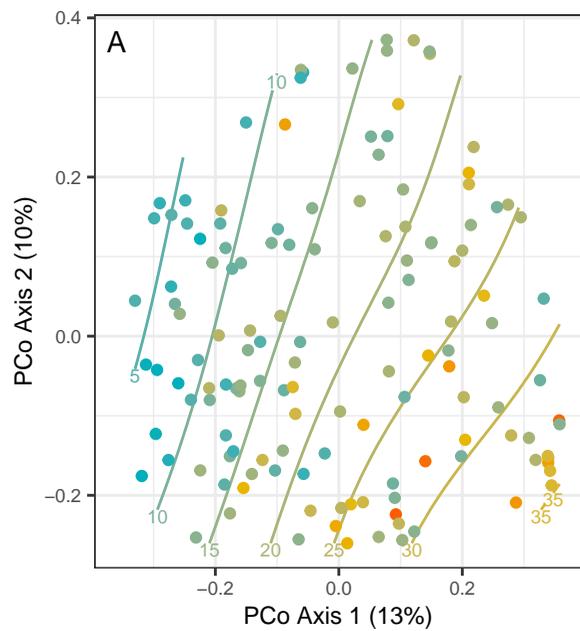
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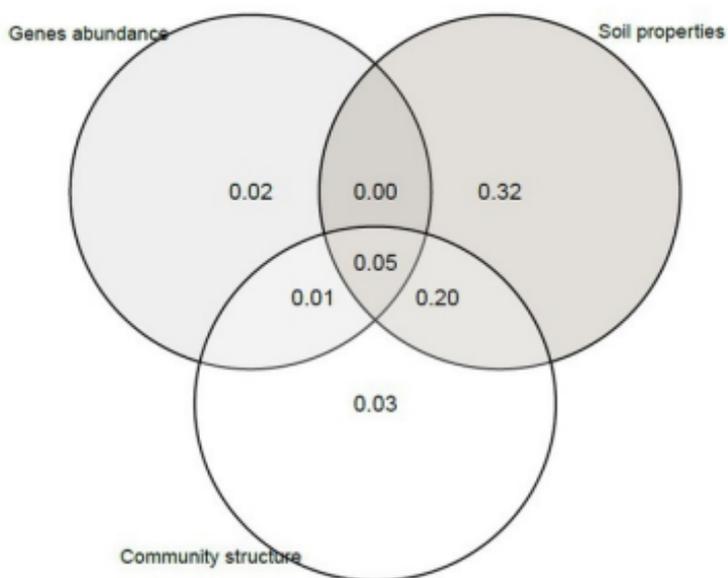
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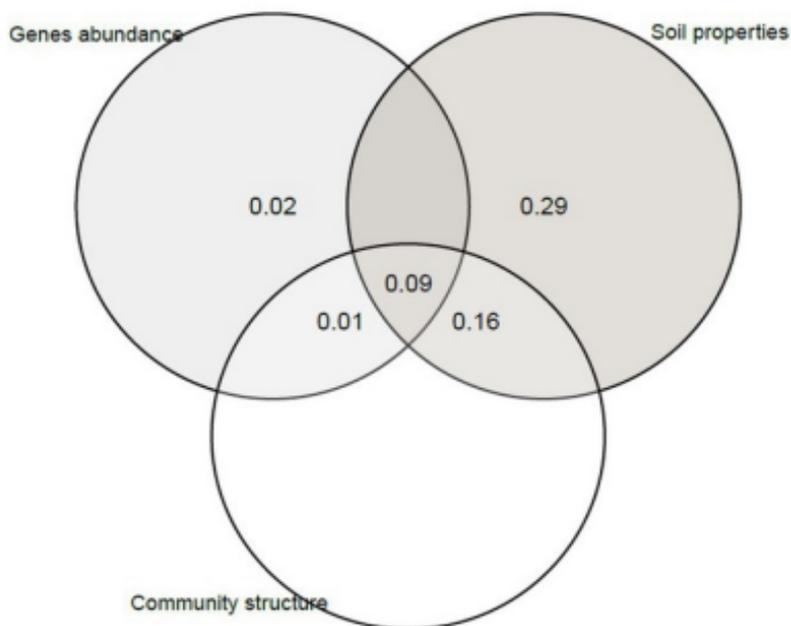




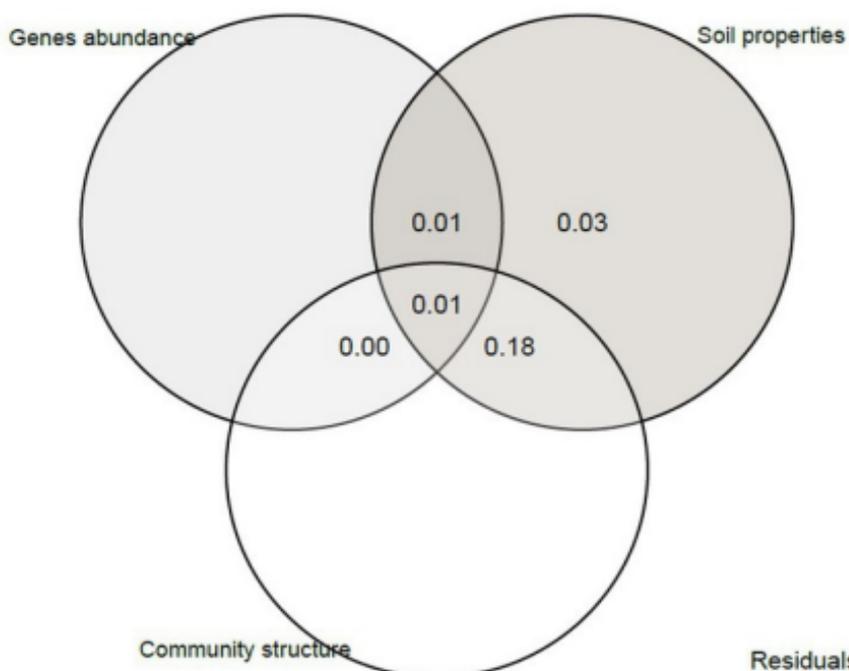


A

Residuals = 0.37

B

Residuals = 0.44

C

Residuals = 0.77