



## **Spatial zoning of microbial functions and plant-soil nitrogen dynamics across a riparian area in an extensively grazed livestock system**

de Sousa, Laura; Glanville, Helen; Marshall, Miles; Williams, Arwel; Abadie, Maïder; Clark, Ian M.; Blaud, Aimeric; Jones, Davey L.

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1 **Spatial zoning of microbial functions and plant-soil nitrogen dynamics across a**  
2 **riparian area in an extensively grazed livestock system**

3

4 Laura L. de Sosa<sup>a,\*</sup>, Helen C. Glanville<sup>a,b</sup>, Miles R. Marshall<sup>c</sup>, A. Prysor Williams<sup>a</sup>,  
5 Maïder Abadie<sup>d</sup>, Ian M. Clark<sup>d</sup>, Aimeric Blaud<sup>d</sup>, Davey L. Jones<sup>a</sup>

6 <sup>a</sup> *School of Environment, Natural Resources & Geography, Bangor University, Deiniol*  
7 *Road, Bangor, Gwynedd, LL57 2UW, United Kingdom*

8 <sup>b</sup> *School of Geography, Geology and the Environment, Keele University, Keele,*  
9 *Staffordshire, ST5 5BG, United Kingdom*

10 <sup>c</sup> *Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Rd, Bangor,*  
11 *Gwynedd, LL57 2UW, United Kingdom*

12 <sup>d</sup> *Department of Sustainable Agriculture Sciences, Rothamsted Research, West Common,*  
13 *Harpenden, Hertfordshire, AL5 2JQ, United Kingdom*

14

15 \* Corresponding author. School of Environment, Natural Resources & Geography,  
16 Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, United Kingdom.

17 *E-mail address:* afs411@bangor.ac.uk (L.L. de Sosa).

18 **ABSTRACT**

19 Anthropogenic activities have significantly altered global biogeochemical nitrogen  
20 (N) cycling leading to major environmental problems such as freshwater eutrophication,  
21 biodiversity loss and enhanced greenhouse gas emissions. The soils in the riparian  
22 interface between terrestrial and aquatic ecosystems may prevent excess N from entering  
23 freshwaters (e.g. via plant uptake, microbial transformations and denitrification).  
24 Although these processes are well documented in intensively managed agroecosystems,  
25 our understanding of riparian N removal in semi-natural systems remains poor. Our aim  
26 was to assess the spatial zoning of soil microbial communities (PLFA), N cycling gene  
27 abundance (archaeal and bacterial *amoA*, *nifH*, *nirK*, *nirS*, *nosZ*), N processing rates and  
28 plant N uptake across an extensively sheep grazed riparian area. As expected, soil  
29 properties differed greatly across the riparian transect, with significant decreases in  
30 organic matter,  $\text{NH}_4^+$ , carbon (C) and N content closest to the river (< 10 m). In addition,  
31 different microbial community structures were found along the transect. The abundance  
32 of N fixation (*nifH*) increased with distance from the river (> 10 m), while ammonia  
33 oxidising archaea (AOA) increased in abundance towards the river.  $\text{N}_2\text{O}$  emissions rates  
34 were limited by C and to a lesser extent by N with greater emissions close to the river.  
35 Plant uptake of urea-derived  $^{15}\text{N}$  was high (ca. 55-70% of that added to the soil) but 30-  
36 65% of the N was potentially lost by denitrification or leaching. Percentage recovered  
37 also suggests that the spatial patterning of plant and microbial N removal processes are  
38 different across the riparian zone. Our study provides novel insights into the underlying  
39 mechanisms controlling the spatial variability of N cycling in semi-natural riparian  
40 ecosystems.

41

42 *Keywords:* buffer strip, ecosystem services, DON, nitrification, heathland, wetlands.

## 43 **1. Introduction**

44           The overuse of nitrogen (N) fertilizers, alongside land use change, has caused the  
45 N saturation of many terrestrial ecosystems worldwide (Gruber and Galloway, 2008).  
46 Further, the resultant N loss from agroecosystems is contributing to many major  
47 environmental problems such as marine and freshwater eutrophication, loss of  
48 biodiversity, climate change and ecosystem acidification (Canfield et al., 2010; Erisman,  
49 2013). Strategies are therefore needed to better retain, or sustainably remove, excess N  
50 from land under agricultural production. One potential mechanism is the active  
51 management of riparian areas at field margins to intercept and mitigate excess N from  
52 migrating towards freshwaters (Mayer et al., 2007). Within these areas, a range of  
53 interrelated biotic and abiotic processes may be involved in N attenuation, including  
54 nitrification, denitrification, mineralization, plant and microbial uptake, mass  
55 flow/diffusion and sorption-desorption (Matheson et al., 2002; Vyzamal, 2007). The  
56 importance of each process, however, is expected to vary greatly between ecosystems and  
57 also from the landscape down to the micrometre scale within the plant-microbial-soil  
58 system (Burt et al., 1999; Sanchez-Pérez et al., 2003).

59           Denitrification has been shown to be of particular importance for riparian wetland  
60 biogeochemistry because of the predominance of anoxic conditions, high concentrations  
61 of dissolved organic carbon (DOC) and the high rates of N fixation (Groffman and  
62 Hanson, 1997). It also represents the ultimate removal mechanism for reactive nitrogen  
63 (e.g.  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{N}_2\text{O}$ ) from terrestrial and aquatic ecosystems (Seitzinger et al., 2006;  
64 Jacinthe and Vidon, 2017). In some cases, however, complete denitrification to  $\text{N}_2$  may  
65 not occur due to a lack of  $\text{N}_2\text{O}$  reductase in the microbial community or if certain  
66 environmental conditions remain sub-optimal (e.g. soil moisture,  $\text{O}_2$  content), leading to  
67 the potential release of environmentally damaging  $\text{N}_2\text{O}$  (Butterbach-Bahl et al., 2013).

68 Additionally, denitrification is strongly coupled, both spatially and temporally, with other  
69 environmental processes such as N fixation, nitrification and anaerobic ammonium  
70 oxidation (anammox) (Vyzamal, 2007; Groffman et al., 2009).

71 To optimise N removal by riparian areas and to implement active management,  
72 requires a good understanding of the key factors which regulate N cycling across these  
73 zones. Fundamental to this, is understanding the spatial abundance and behaviour of the  
74 underlying microbial communities which control how and when the different N  
75 transformations occur (Herbert, 1999; Chon et al., 2011). In this respect, few studies have  
76 tried to combine the analysis of key N cycling genes (abundance and transcription) and  
77 quantification of  $\text{N}_2\text{O}:\text{N}_2$  production to gain a better insight into the spatio-temporal  
78 factors regulating  $\text{N}_2\text{O}$  fluxes (Avrahamia and Bohannan, 2009)). However, contradictory  
79 studies showing a clear relationship between gene copy number and  $\text{N}_2\text{O}$  emission rates  
80 or a total lack of it, are commonly presented, highlighting the need for further research in  
81 this area (Bakken et al., 2012; Di et al., 2014). Additionally, research in wetland  
82 biogeochemistry has frequently focused on single-ecosystem processes (i.e.  
83 denitrification) rather than providing a more holistic view of microbial community  
84 functioning (Gutknecht et al., 2006). Therefore, there is a need to improve our  
85 understanding of the links (from genes to ecosystems) between physical, biogeochemical  
86 and ecological processes that drive the services of freshwater systems

87 Alongside the microbial community, wetland vegetation also plays a major role  
88 in regulating N losses via denitrification (Schnabel et al., 1996; Veraart et al., 2011). For  
89 example, plants can alter the size and composition of the soil microbial community,  
90 stimulate microbial activity via C rhizodeposition, and change soil oxidation status  
91 (Nijburg et al., 1997; Tabuchi et al., 2004; Groffman et al., 2009). In addition, wetland  
92 plants employ numerous physiological adaptations to overcome anoxia in waterlogged

93 soils including: shallow rooting, dumping of respiratory by-products into the rhizosphere  
94 (e.g. lactic acid) and the formation of aerenchyma (Wheeler, 1999). In light of this, the  
95 choice of plant species is likely to be very important for improved riparian management  
96 and freshwater protection.

97         While much work has been undertaken on N removal in riparian areas adjacent to  
98 intensive cropping systems, comparatively little work has been undertaken in extensively  
99 grazed livestock systems (Wells et al., 2016). In these systems, urine hotspots represent  
100 the major input of reactive N and are expected to greatly modify soil microbial  
101 communities involved in N cycling (Di et al., 2010). In this context, the main objectives  
102 of the present study were: (1) to gain further insight into the environmental factors  
103 controlling riparian soil N cycling and how they contribute to explaining the spatial and  
104 temporal variability of N cycling in semi-natural ecosystems; (2) to estimate the role of  
105 different vegetation communities in N uptake across the riparian zone; and (3) to link N  
106 cycling gene abundance to N removal processes.

107

## 108 **2. Materials and methods**

### 109 *2.1. Study site*

110         The experimental site was located in the upper, southern area of the Conwy  
111 catchment, North Wales, UK (52° 59' 8.90"N, 3° 49' 15.99"W; Fig. 1; Figs. S1 and S2).  
112 The study area has been classified as blanket bog according to the New Phase 1 habitat  
113 survey (Lucas et al., 2011) and considered a Special Area of Conservation (SAC) under  
114 the EC Habitats Directive (94/93/EEC). The climate of the upper reaches of the Conwy  
115 catchment is characterized by relatively high rainfall and cool temperatures (mean annual  
116 rainfall of 2180 mm and mean annual soil temperature at 30 cm depth is 8 °C; based on  
117 30-year average 1981-2010 data from the UK Met Office). The area was subject to sheep

118 (*Ovis aries* L.) grazing at a low stocking density (0.1 ewe ha<sup>-1</sup>). A detailed description of  
119 the Conwy catchment and land use can be found in Emmett et al. (2016) and Sharps et al.  
120 (2017).

121

## 122 2.2. Sampling strategy

123 Four 25 m long transects, 5-10 m apart, and perpendicular to a headwater stream of  
124 the Conwy River, were delineated for sampling during the month of October 2016 (Fig.  
125 2). The maximum length of the transects was decided according to the extent of the  
126 riparian zone as defined by the variable buffer delineation method (de Sosa et al., 2017).  
127 Intact soil cores (5 cm diameter, 0-15 cm depth) were collected at three different zones  
128 (from this point onwards in the manuscript, these are referred to as zones 1, 2 and 3),  
129 selected according to their dominant vegetation cover (Fig. 2). Zone 1 was dominated by  
130 thick tufts of soft rush (*Juncus effusus* L.) and located < 5 m to the river. Zone 2  
131 corresponded to the transitional area between the grasses and the heathland (5-10 m) and  
132 zone 3 (> 10 m) represented the area dominated by typical peat-forming heathland species  
133 such as bog-mosses (*Sphagnum* spp.), *Calluna vulgaris* (L.) Hull, *Erica tetralix* L. and  
134 *Scirpus cespitosus* L. (Fig. S1-S2). Along each transect, two sample points were located  
135 within zone 1 (2 and 5 m from the edge of the river), one sample point was located within  
136 zone 2 (5-10 m), and two sampling points were located in zone 3 (i.e. 15 and 25 m; Fig.  
137 2).

138 Intact soil cores were taken with a Russian auger (5 cm diameter, 15 cm in length;  
139 Eijkelkamp Soil & Water, Giesbeek, The Netherlands) to conduct the main denitrification  
140 experiment. Additional intact soil cores were taken for analysis of soil physicochemical  
141 properties prior to conducting the laboratory study and a further 20 cores for bulk density  
142 determination. All soil samples were stored at 4 °C prior to analysis except for subsamples

143 (~25 g) which were used for Phospholipid Fatty Acid analysis (PLFA) and DNA  
144 extractions. These samples were stored immediately at -80 °C.

145

### 146 2.3. General soil characterization

147 Soil samples were passed through a 2 mm sieve to remove any plant material and  
148 to ensure sample homogeneity. They were held at field moisture for all subsequent  
149 analyses to represent field conditions. Soil water content was determined gravimetrically  
150 (24 h, 105 °C) and soil organic matter content was determined by loss-on-ignition (LOI)  
151 (450 °C, 16 h). Soil pH and electrical conductivity (EC) were measured using standard  
152 electrodes in a 1:2.5 (*w/v*) soil-to-deionised water mixture. Total available ammonium  
153 (NH<sub>4</sub>-N) and nitrate (NO<sub>3</sub>-N) in soil were determined within 0.5 M K<sub>2</sub>SO<sub>4</sub> extracts (1:5  
154 *w/v*) via the colorimetric salicylate procedure of Mulvaney (1996) and the vanadate  
155 method of Miranda et al. (2001), respectively. Available phosphate (P) was quantified  
156 with 0.5 M acetic acid extracts (1:5 *w/v*) following the ascorbic acid-molybdate blue  
157 method of Murphy and Riley (1962) and total C (TC) and N (TN) were determined with  
158 a TruSpec<sup>®</sup> elemental analyser (Leco Corp., St Joseph, MI). Dissolved organic C (DOC)  
159 and total dissolved N (TDN) were quantified in 1:5 (*w/v*) soil-to-0.5 M K<sub>2</sub>SO<sub>4</sub> extracts  
160 (Jones and Willett, 2006) using a Multi N/C 2100 TOC analyzer (AnalytikJena, Jena,  
161 Germany). Total soil porosity was determined using the equation of 1-(bulk  
162 density/particle density for organic soils) and percent water-filled pore space (WFPS) was  
163 obtained from the relationship between the volumetric water content and total soil  
164 porosity. Anaerobic mineralizable N (AMN) was determined by the anaerobic incubation  
165 of soil samples for 14 days at 25-30 °C in the dark, followed by extraction with 1 M KCl  
166 and measurement of NH<sub>4</sub>-N produced as described above (Bundy and Meisinger, 1994).  
167 Anaerobically mineralizable organic C (AMOC) was calculated as described in Ullah and

168 Faulkner (2006). Briefly, moist soil samples were placed in gas-tight containers and  $\text{NO}_3^-$   
169 was added to remove any soil limitation. Containers were purged with  $\text{N}_2$  gas to induce  
170 anoxic conditions and stored in the dark at room temperature (25 °C). The headspace of  
171 the containers was sampled after 1, 24, 48 and 72 h of incubation and analysed for  $\text{CO}_2$   
172 concentration on a Clarus 500 gas chromatograph with a TurboMatrix headspace  
173 autoanalyzer (Perkin-Elmer Inc., Waltham, CT).

174

#### 175 *2.4. Phospholipid fatty acid analysis*

176 Microbial community structure was measured by phospholipid fatty acid (PLFA)  
177 analysis following the method of Buyer and Sasser (2012). Briefly, samples (2 g) were  
178 freeze-dried and Bligh-Dyer extractant (4.0 ml) containing an internal standard added.  
179 Tubes were sonicated in an ultrasonic bath for 10 min at room temperature before rotating  
180 end-over-end for 2 h. After centrifuging (10 min) the liquid phase was transferred to clean  
181 13 mm × 100 mm screw-cap test tubes and 1.0 ml each of chloroform and water added.  
182 The upper phase was removed by aspiration and discarded while the lower phase,  
183 containing the extracted lipids, was evaporated at 30 °C. Lipid classes were separated by  
184 solid phase extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per  
185 well (Phenomenex, Torrance, CA). Phospholipids were eluted with 0.5 ml of 5:5:1  
186 methanol:chloroform: $\text{H}_2\text{O}$  (Findlay, 2004) into glass vials, the solution evaporated (70  
187 °C, 30 min). Transesterification reagent (0.2 ml) was added to each vial, sealed and  
188 incubated (37 °C, 15 min). Acetic acid (0.075 M) and chloroform (0.4 ml each) were then  
189 added. The chloroform was evaporated just to dryness and the samples dissolved in  
190 hexane. The samples were analysed with a 6890 gas chromatograph (Agilent  
191 Technologies, Wilmington, DE) equipped with autosampler, split-splitless inlet, and  
192 flame ionization detector. Fatty acid methyl esters were separated on an Agilent Ultra 2

193 column, 25 m long  $\times$  0.2 mm internal diameter  $\times$  0.33  $\mu$ m film thickness. Standard  
194 nomenclature was followed for fatty acids (Frostegård et al., 1993). A detailed description  
195 of PLFA markers and taxonomic microbial groups is provided in Table S1.

196

## 197 *2.5. Denitrification and potential N<sub>2</sub>O emissions*

198 Denitrification rates were measured using the acetylene (C<sub>2</sub>H<sub>2</sub>) block method based  
199 on the intact core technique developed by Tiedje et al. (1989). Although this technique  
200 presents limitations such as the poor diffusion of C<sub>2</sub>H<sub>2</sub> into the soil, it has been found to  
201 produce similar results to experiments using <sup>15</sup>N tracers (Aulakh et al., 1991).

202 In brief, intact soil cores (approximately 37  $\pm$  1.5 g dry weight soil) were placed in  
203 PVC tubes (10  $\times$  15 cm) to maintain soil structure. These tubes were then placed in gas-  
204 tight containers (1.4 dm<sup>3</sup> volume; Lock & Lock Ltd., Seoul, Republic of South Korea).

205 To measure denitrification, 20 ml of 4 different C and N amendments were applied  
206 to individual soil cores ( $n = 20$  per amendment):

- 207 1) Control (distilled water addition only)
- 208 2) Glucose-C addition (glucose solution containing 4 g C l<sup>-1</sup>; 55 mM glucose)
- 209 3) Urea-N addition (artificial sheep urine containing 2 g N l<sup>-1</sup>; Selbie et al., 2015)
- 210 4) Urea-N + glucose-C addition (artificial urine plus glucose solution containing 2  
211 g N l<sup>-1</sup> and 4 g C l<sup>-1</sup> respectively).

212 Urea was selected as it represents one of the main N inputs to upland grazed ecosystems.  
213 The N concentration was chosen according to the concentration range in urine under a  
214 light grazing regime (Selbie et al., 2015). The ratio of C-to-N was chosen based on  
215 experimental values presented in Her and Huang (1995). Glucose was chosen as it  
216 represents a labile C substrate that can be utilized by almost all soil microorganisms

217 (Gunina and Kuzyakov, 2015). The concentration of added C also reflects a typical sugar  
218 concentration that would occur in soil upon root cell lysis (Jones and Darrah, 1996).

219 All cores were directly injected with 5 ml of C<sub>2</sub>H<sub>2</sub> into the middle of the soil  
220 volume. The cores were then placed into gas-tight containers and 10% of the headspace  
221 replaced with C<sub>2</sub>H<sub>2</sub> to block the conversion of N<sub>2</sub>O to N<sub>2</sub> gas. The control cores were only  
222 amended with 20 ml of distilled water without C<sub>2</sub>H<sub>2</sub> addition. The containers were stored  
223 at 10 °C in the dark to prevent C<sub>2</sub>H<sub>2</sub> breakdown. Headspace gas was sampled at 0, 2, 6  
224 and 24 h and stored in pre-evacuated 20 ml glass vials before being analysed for N<sub>2</sub>O  
225 concentration on a Clarus 500 gas chromatograph with a TurboMatrix headspace  
226 autoanalyzer (Perkin-Elmer Inc., Waltham, CT). Prior to gas sampling, the headspace was  
227 homogenised by gently mixing with a syringe. At the end of the experiment, each  
228 individual core was weighed and N<sub>2</sub>O fluxes corrected accordingly. The rate of N<sub>2</sub>O  
229 production was calculated in µg N-N<sub>2</sub>O g<sup>-1</sup> dw h<sup>-1</sup>. Cumulative N<sub>2</sub>O emissions were  
230 calculated by integration using the trapezoidal rule.

231

## 232 *2.6. Nitrogen uptake by vegetation*

233 The role of vegetation in N uptake was measured in the field using <sup>15</sup>N-labelled  
234 urine. Two independent sets of plots (50 cm times 50 cm) were randomly selected within  
235 each replicate vegetation zone, one set received no N additions (herein referred to as the  
236 control set) while the second received <sup>15</sup>N-labelled artificial urine.

237 Prior to addition of the <sup>15</sup>N-labelled treatment, turfs (20 cm times 20 cm) and  
238 associated soil (0-15 cm depth) were taken from the centre of each of the control plots to  
239 obtain <sup>15</sup>N natural abundances for each plant and soil component. After harvest, the  
240 samples were transferred to the laboratory and separated into soil, roots, shoots and  
241 mosses for <sup>15</sup>N determination. Subsequently, in each <sup>15</sup>N-labelling plot, 250 ml of

242 artificial urine labelled with  $^{15}\text{N}$  urea (15 atom %) at a rate of  $2 \text{ g N l}^{-1}$  was applied  
243 (equivalent to  $20 \text{ kg N ha}^{-1}$ ). Ten pulses of  $^{15}\text{N}$ -labelled urine (each pulse was 25 ml in  
244 volume) were injected with a syringe (0.84 mm bore  $\times$  5 cm long) into the soil underneath  
245 the plants (0-15 cm depth) in the centre of the plot. Depth of urine injection was selected  
246 according to previous observations of urine infiltration into the soil. The volume and  
247 concentration of N added followed that of a typical sheep urine event (Marsden et al.,  
248 2016). Immediately after the final  $^{15}\text{N}$  pulse addition, the area was protected with  
249 individual wire mesh cages to prevent livestock trampling and grazing. One week after  
250  $^{15}\text{N}$  addition, a  $20 \times 20 \text{ cm}^2$  turf and associated soil (0-15 cm depth) was harvested from  
251 the middle of each plot, transferred to the laboratory and separated into soil and plant  
252 components as described above for  $^{15}\text{N}$  determination.

253 Soil for  $^{15}\text{N}$  analysis was passed through a 2-mm sieve and subsamples (ca. 40 g)  
254 were oven-dried (48 h,  $80 \text{ }^\circ\text{C}$ ) before being weighed and ground for  $^{15}\text{N}$  analysis. Plant  
255 shoot and root material followed the same drying procedure after being washed with  
256 distilled water to remove any exogenous isotope label. The same procedures were  
257 followed for the control samples one week before to avoid any cross-contamination with  
258 the  $^{15}\text{N}$ -urea labelled samples. All fractions were analysed separately for  $\delta^{15}\text{N}$  at the UC  
259 Davis Stable Isotope Facility (UC Davis, Davis, CA). Values of  $^{15}\text{N}$  are presented directly  
260 as the atom% of  $^{15}\text{N}$  in the sample. The  $^{15}\text{N}$  atom% excess was calculated as the  $^{15}\text{N}$   
261 atom% difference between enriched samples and values of background natural  
262 abundances (control). Recovery of tracer  $^{15}\text{N}$  (%) was calculated by multiplying the N  
263 content in the pool by its mass per square meter and  $^{15}\text{N}$  atom% excess divided by total  
264 added  $^{15}\text{N}$  per square meter (Xu et al., 2011).

265

266 *2.7. DNA extraction and quantitative PCR*

267 A subsample of soil (ca. 25 g) was taken from each of the cores used for  
268 physicochemical analysis and stored at -80 °C prior to DNA extraction. The DNA was  
269 extracted from three 250 mg subsamples using an UltraClean<sup>®</sup> Microbial DNA Isolation  
270 Kit (Mo Bio Laboratories Inc., Carlsbad, CA) following the manufacturer's instructions.  
271 Triplicate DNA extractions for each soil sample were pooled together to give a total  
272 volume of 150 µl. Extractions of DNA were concentrated to give a final volume of 50 µl  
273 using a Savant SVC100H SpeedVac Concentrator (ThermoFisher Scientific Inc.,  
274 Waltham, MA). Extracted DNA was visualized by 0.9% agarose gel electrophoresis and  
275 nucleic acid staining with SafeView<sup>®</sup> (NBS Biologicals, Huntingdon, UK). The  
276 concentrations of DNA were checked using Quant-iT<sup>™</sup> dsDNA Assay Kit  
277 (ThermoFisher). Samples were then stored at -80 °C prior to further analysis.

278 Microbial N cycling gene abundance was investigated by quantitative-PCR  
279 (qPCR) targeting specific genes or genetic regions. Bacterial and archaeal communities  
280 were targeted via the 16S rRNA genes, while the fungal community abundance by the  
281 ITS region. The different communities involved in N-cycling were investigated: N  
282 fixation (*nifH* gene); nitrification by targeting the ammonia oxidising bacteria (AOB) and  
283 archaea (AOA) (*amoA* gene), and denitrifiers via the nitrite reductase (*nirK* and *nirS*  
284 genes) and the nitrous oxide reductase (*nosZ* genes clade I and II) (Table S2).

285 Quantitative-PCR amplifications were performed in 10 µl volumes containing 5  
286 µl of QuantiFast (Qiagen, Manchester, UK), 2.8 µl of nuclease-free water (Severn  
287 Biotech, Kidderminster, UK), 0.1 µl of each primer (1 µM) and 2 µl of template DNA at  
288 5 ng µl<sup>-1</sup>, using a CFX384 Touch<sup>®</sup> Real-Time PCR Detection System (Bio-Rad, Hemel  
289 Hempstead, UK). The standards for each molecular target were obtained using a 10-fold  
290 serial dilution of PCR products amplified from an environmental reference DNA (also  
291 used as positive control) and purified by gel extraction using the Wizard<sup>®</sup> SV Gel and

292 PCR Clean Up System (Promega, Southampton, UK) following the manufacturer's  
293 instruction and quantified by fluorometer Qubit<sup>®</sup> 2.0 dsDNA BR Assay Kit (Thermo  
294 Fisher Scientific). Standard curve template DNA and the negative/positive controls were  
295 amplified in triplicate. Amplification conditions for all qPCR assays consisted in 2 steps:  
296 first denaturation at 95 °C for 5 min followed by 40 cycles at 95 °C for 10 s and 60 °C  
297 for 30 s that included annealing, elongation and reading. Each amplification was followed  
298 by melting curve (increase in temperature from 60 °C to 95 °C, with a reading every 0.5  
299 °C) to assess the specificity of each assay. The efficiency of the qPCR varied between  
300 81.5% and 94.5%, and  $r^2$  between 0.996 and 0.999. The melting curves showed specificity  
301 for all the genes, except as expected for the fungal ITS, that showed the amplification of  
302 products of different lengths, due to the variability in length of the ITS region between  
303 different fungal taxa (Manter and Vivanco, 2007).

304

## 305 *2.8. Statistical analysis*

306 Statistical analysis was performed with SPSS v22 for Windows (IBM Corp.,  
307 Armonk, NY). All data were analysed for normality and homogeneity of variance with  
308 Shapiro Wilk's tests and Levene's statistics, respectively. Transformations ( $\log_{10}$  or  
309 square root) to accomplish normality and homogeneity of variance were done when  
310 necessary (i.e. bulk density, available P, microbial biomass PLFA, <sup>15</sup>N recovery, and  
311 cumulative N<sub>2</sub>O, untransformed values are presented). For all statistical tests,  $P < 0.05$  was  
312 selected as the significance cut-off value. Analysis of variance (one-way ANOVA) was  
313 performed to explore the difference of soil physicochemical properties, gene copy  
314 numbers, PLFA ratios of microbial groups respective to distance from the river followed  
315 by Tukey's post-hoc test to assess differences across the riparian transect. Principal  
316 component analysis (PCA) was used to explore the spatial relationships of PLFA

317 microbial groups (%) relative to distance from the river. Cumulative N<sub>2</sub>O emissions after  
318 treatment application across the riparian transect were compared by Welch's test followed  
319 by Games-Howell post-hoc test, due to the data not conforming to homogeneity of  
320 variance even after data transformation. In contrast, a one-way ANOVA followed by  
321 Tukey's post-hoc test was performed to assess differences in cumulative N<sub>2</sub>O emissions  
322 between treatments for each sampling distance from the river (i.e. 2, 5, 10, 15 and 25 m).  
323 Two separate analyses were conducted to explore differences in <sup>15</sup>N recovery due to the  
324 data not conforming to homogeneity of variance even after data transformation. A one-  
325 way ANOVA and Tukey's post-hoc test was performed to explore differences in the  
326 percentage allocation of <sup>15</sup>N to the different fractions (e.g. shoots, roots, moss and soil)  
327 across the different riparian zones. A second one was used to assess how <sup>15</sup>N recovery  
328 differed within each specific fraction across the three zones. A mixed model was also  
329 performed with distance from the river as a fixed effect and transect as a random effect  
330 to assess <sup>15</sup>N recovery and gene copy number across the riparian zone, but the results did  
331 not differ from ANOVA, and only the ANOVA results is presented in the article

332 Spearman rank correlation coefficients ( $\rho$ ) were used to evaluate the relationship  
333 between soil physicochemical properties and cumulative N<sub>2</sub>O emissions, gene copy  
334 number, or PLFA biomarkers ratio whereas linear regressions ( $r^2$ ) were used between soil  
335 physicochemical properties and PLFA biomarker ratios.

336

### 337 **3. Results**

#### 338 *3.1. General soil characterization*

339 Significant differences in all soil properties, except for NO<sub>3</sub><sup>-</sup> and total dissolved N  
340 concentration and anaerobic mineralizable N (AMN), were found across the riparian  
341 transect relative to distance from the main river channel (Table 1). Zone 2 showed an

342 increase in pH values by 0.66-0.85 unit in comparison with zone 1 and zone 3. Likewise,  
343 EC was approximately 2-fold greater in zone 1 and 3 relative to zone 2. In addition, soil  
344 organic matter (SOM) tended to increase with distance from the river being 60% higher  
345 at the distal points (15 and 25 m) compared with those closer to the river. The high SOM  
346 levels associated with soils furthest away from the river contributed to lower bulk  
347 densities, higher soil porosities and increased soil water content. Available  $\text{NH}_4^+$   
348 concentrations were 3.6 greater in soil from zone 3 in comparison with zone 1 and 1.8  
349 times greater than the soil in zone 2, while  $\text{NO}_3^-$  did not show any significant differences.  
350 Similarly, available P was 10-times greater in zone 3 relative to zones 1 and 2. Total C,  
351 total N and the C-to-N ratio were greater in zone 3 relative to zones 1 and 2 and a similar  
352 trend was also observed for DOC.

353 Anaerobic incubation of soils across the transect showed that the amount of AMOC  
354 in zone 3 was significantly greater than in zone 1 and 2 (~ 3 and 1.5 times, respectively)  
355 (Table 1). In contrast, AMN showed little trend across the transect.

356

### 357 *3.2 Microbial community structure and abundance*

358 Microbial biomass determined from total PLFA content showed a general decline  
359 across the riparian transect towards the river channel. Principal Component Analysis  
360 (PCA) of PLFA microbial groups (% abundance) across the transect explained 72.6% of  
361 the total variance within the dataset on the first two principal components (PC) (Fig. 3).  
362 The spatial segregation of cluster centroids within the PCA indicates that in zone 1 the  
363 most influential components were anaerobes and putative arbuscular mycorrhizal fungi  
364 (AM fungi). In contrast, Gram (+) and Gram (-) bacteria were the dominant groups in  
365 zone 2 and 3, respectively. Zone 2 showed the greatest microbial variability.

366 The fungi/bacteria ratio decreased by 2 to 2.5 times from zone 1 to zone 2 and 3  
367 (Table S3,  $P = 0.008$ ). The ratio of Gram (+)/Gram (-) was over 2 times greater in zone 2  
368 than zone 3 but it did not differ from zone 1 ( $P = 0.001$ ). On average, 16w/17cyclo and  
369 18w/19cyclo ratio (indicative of an actively growing community under low stress  
370 conditions) was 2.5-fold greater in zone 3 than zone 1 and 2 (Table S3,  $P < 0.0001$ ). There  
371 were highly positive relationships between fungi/bacteria ratio with bulk density and  
372 negatively with total porosity and soil water content ( $r^2 = 0.60$ ,  $P < 0.001$  for bulk density  
373 and total porosity,  $r^2 = 0.45$ ,  $P = 0.001$ , soil water) whereas Gram (+)/Gram (-) ratio was  
374 negatively correlated to  $\text{NH}_4\text{-N}$ , soil water content, SOM, available-P and DOC content  
375 ( $r^2 > 0.68$  for available P and DOC,  $r^2 > 0.53$  the rest,  $P < 0.001$  in all cases). In contrast,  
376 a positive correlation was found between 16w/17cyclo and 18w/19cyclo ratios and DOC,  
377 soil water, C-to-N ratio and SOM content ( $r^2 > 0.61$ , for DOC,  $r^2 > 0.71$ , for soil water  
378 and C-to-N ratio,  $r^2 > 0.82$ , for SOM,  $P < 0.001$  in all cases).

379 The archaeal 16S rRNA gene abundance tended to increase with distance from the  
380 river but the results were not significantly different ( $P > 0.05$ ) (Fig. 4). In contrast, the  
381 fungal ITS region abundance showed the opposite trend but was also not significant. The  
382 bacterial 16S rRNA gene abundance displayed on average 2 times greater bacterial copies  
383 in zone 2 than the distal area but it was not significant (Fig. 4).

384 Significant positive correlations were found between bacterial *16SrRNA* and pH  
385 and EC ( $\rho = 0.48$ ,  $-0.45$ , respectively) whereas archaeal *16SrRNA* correlated negatively  
386 with soil bulk density and positively with total porosity ( $\rho = -0.57$ ,  $0.56$ , respectively;  
387 Table 2).

388

389 3.2.  $^{15}\text{N}$  uptake by the vegetation

390 No significant differences were found between the recovery of  $^{15}\text{N}$  in the different  
391 plant and soil fractions across the riparian transect (zone 1, 2 and 3;  $P > 0.05$ ). Similar  
392 percentages of total  $^{15}\text{N}$  recovery of added  $^{15}\text{N}$  were obtained for plants and soil in zones  
393 2 and 3 (71.9 % and 79.3%, respectively), whereas only 56.8% was recovered in the plants  
394 and soil within zone 1 although it was not significant (Fig. 5). Generally, there were very  
395 few differences between the amounts of  $^{15}\text{N}$  recovered in the different plant-soil fractions  
396 within each zone, Only in zone 2, were four times more  $^{15}\text{N}$  was recovered in the shoots  
397 compared to the soil ( $P = 0.012$ ; Fig. 5).

398

### 399 3.3. Potential denitrification and $\text{N}_2\text{O}$ emissions

400 In response to the addition of labile C and/or N to the soil, greater cumulative  $\text{N}_2\text{O}$   
401 emissions were only observed within zone 1, showing little or no effect in zones 2 and 3  
402 (Fig. 6). In zone 1, the addition of labile C-only increased  $\text{N}_2\text{O}$  emissions by a factor of  
403 1000, from  $0.004 \pm 0.001$  to  $4.07 \pm 0.14$   $\text{mg N kg}^{-1} \text{ h}^{-1}$  relative to the control in the area  
404 closest to the river (e.g. 2 m,  $P < 0.001$ ). Similarly, the addition of C and N together also  
405 increased  $\text{N}_2\text{O}$  emissions relative to the control ( $0.004 \pm 0.001$  to  $2.95 \pm 0.14$   $\text{mg N kg}^{-1}$   
406  $\text{h}^{-1}$ ) at 2 m from the river. After the addition of labile C alone or in combination with N,  
407 emissions of  $\text{N}_2\text{O}$  were 78 and 45 times higher, respectively than the control at 5 m from  
408 the river (Fig. 6). Although urea-N addition also increased  $\text{N}_2\text{O}$  emissions in zone 1 ( $0.24$   
409  $\pm 0.06$   $\text{mg N kg}^{-1} \text{ h}^{-1}$  at 2 m, and  $0.61 \pm 0.36$   $\text{mg N kg}^{-1} \text{ h}^{-1}$  at 5 m), fluxes were not  
410 significantly different from the control ( $P > 0.05$ ).

411  $\text{N}_2\text{O}$  emissions across the riparian transect significantly differed for all treatments  
412 with respect to the distance from the river ( $P < 0.001$ , treatments with C addition alone  
413 or in combination with N addition;  $P < 0.05$ , urea-N only addition). Basal emissions of  
414  $\text{N}_2\text{O}$  from the control cores did not show significant differences with distance from the

415 river ( $P > 0.05$ ). Carbon-only addition greatly stimulated emissions of  $N_2O$  with distance  
416 from river, with the area closest to the river (2 m) emitting on average 80 times more  $N_2O$   
417 than the distal point of the transect (25 m). The addition of C together with N increased  
418  $N_2O$  emissions at 2 m from the river by 60, 90 and 101% in comparison to the amount  
419 emitted at 5 m and zone 2 and 3, respectively (Fig. 6).

420 Significant positive correlations were found between  $N_2O$  emissions and bulk  
421 density, whereas soil water content, total N, total porosity and AMOC correlated  
422 significantly but negatively with  $N_2O$  production for all treatments except the control  
423 (Table 3).

424

#### 425 3.4. *N* cycling gene abundance

426 Ammonia oxidizing bacteria (AOB) and archaea (AOA) showed different  
427 abundance patterns with respect to distance from the river (Fig. 7). While the proximity  
428 of the river had no effect on the bacterial *amoA* gene numbers, archaeal *amoA* gene copy  
429 number significantly decreased ( $P = 0.001$ ) on average by up to 84% from zone 1 closest  
430 to the river to zone 2 and by 98% with respect to zone 3. The archaeal-to-bacterial *amoA*  
431 gene ratios were approximately 5 and 46-fold greater in zone 1 relative to zone 2 and 3  
432 respectively (Fig. S3). In contrast, the *nifH* gene abundance significantly increased ( $P =$   
433  $0.001$ ) from close to the river to the distal point by 67-82%, whereas a difference with  
434 respect to zone 2 was only found for 2 m. Zone 1, specifically the closest point to the  
435 river, displayed the lowest value for *nirS* gene abundance which represents 3.5 lower  
436 values than zone 2 ( $P = 0.038$ ) (Fig. 7). In contrast, *nirK* and *nosZ* gene copy numbers  
437 did not change significantly across the transect ( $P > 0.05$ ). The clade II of the *nosZ* gene  
438 could not be amplified despite the positive control being amplified (data not shown)

439 Abundance of *nirS* and *nosZ* genes correlated positively with pH ( $\rho \sim 0.5$ ) but  
440 negatively with EC ( $\rho = -0.52, -0.63$ , respectively) (Table 2). A negative correlation was  
441 found between *nifH* and soil bulk density while archaeal *amoA* was correlated positively  
442 with bulk density (Table 2). Significant positive correlations were found between *nifH*  
443 and soil water content, AMOC, total porosity,  $\text{NH}_4^+$  content and microbial PLFA whereas  
444 archaeal *amoA* abundance correlated negatively to the same soil properties (Table 2). The  
445 bacterial *amoA* and *nirS* genes did not show any significant correlations.

446 A positive strong correlation was found between copies of bacterial *16SrRNA*,  
447 bacterial *amoA* and *nirK* ( $\rho > 0.73, P < 0.001$  in all cases) whereas *nifH* showed a highly  
448 positive correlation with *nirK* ( $\rho > 0.52, P = 0.001$ ).

449

## 450 **4. Discussion**

### 451 *4.1. Soil biology and biogeochemistry across the riparian zone*

452 The riparian zone showed distinct spatial patterns in soil properties, despite the  
453 relatively short length of the transect. Results from this study clearly showed that  
454 vegetation, influenced in turn by the prevailing hydrodynamic conditions, had a striking  
455 effect on most of the soil's physicochemical properties. This finding is supported by a  
456 range of studies which have established that mean high water level together with the  
457 frequency of water fluctuation is a critical factor controlling species diversity and  
458 abundance close to watercourses (Wierda et al., 1997; Lou et al., 2016). In our study,  
459 there were lower amounts of soil organic matter and nutrients (N and P) in soils close to  
460 the river in comparison to those further away. These can be ascribed to differences in  
461 erosion-depositional processes occurring along the transect. Alongside differences in  
462 water table depth, this has led to the formation of two very distinct vegetation  
463 communities: one that contains species that can tolerate extreme waterlogging and anoxia

464 (via aerenchyma formation and organic acid excretion) and high levels of exogenous Fe<sup>2+</sup>  
465 and Mn<sup>2+</sup> (e.g. *Juncus effusus*; Visser et al., 2006; Blossfeld et al., 2011), and another that  
466 relies on obligate aerobic symbionts, which lacks aerenchyma and can only tolerate mild  
467 hypoxia (e.g. *Calluna* heathland; Gerdol et al., 2004; Rydin and Jeglum, 2013). These  
468 differences in plants are likely to be a key driver in shaping rhizosphere microbial  
469 communities and the dominant N cycling pathways.

470         The microbial community structure was different in the three riparian zones due  
471 the distinct soil physicochemical properties, and plant cover that are highly dependent on  
472 local hydrological regime (Gutknecht et al., 2006; Balasooriya et al., 2007). For example,  
473 the fungal-to-bacterial ratio was very low indicating a clear dominance of bacteria  
474 community over fungi. Nevertheless, the higher ratios in areas close to the river suggests  
475 a zonation pattern in fungal communities across the transect, probably linked to plant type  
476 and poor nutrient conditions (Bohrer et al., 2004; Six et al., 2006). The Gram (+)/Gram  
477 (-) ratio decreased in zone 3 ( $\geq 15$  m) in relation to the increase in SOM, total C and N  
478 content. Gram (-) bacteria are thought to be copiotrophic organisms with a high growth  
479 rate, using labile substrate such as in zone 3, while Gram (+) bacteria are thought to be  
480 oligotrophic organisms that are better decomposers of less labile soil organic matter but  
481 have a lower growth rate (Fierer et al., 2007). Furthermore, the greater relative abundance  
482 of cyclopropanes close to the river (64% more than distal areas), which indicates the  
483 growth rate in the bacterial community and has been linked to changes in nutrient  
484 availability, infers that the most rapid growth or turnover rates will occur in distal areas  
485 of the river as a result of higher nutrient availability and lower stress conditions (i.e. water  
486 fluctuation) (Ponder and Tادros, 2002; Bossio et al., 2006).

487

488 *4.2. N cycling across the riparian transect*

489           The balance between the different steps of the N cycle varied along the riparian  
490 transect, while the plant and soil retention potential was constant, showing the varying  
491 potential of riparian wetland for N attenuation. The amount of N added did not exceed N  
492 plant demand, however, the total higher plant recovery of  $^{15}\text{N}$  (ca. 30-40%) indicated a  
493 relatively high rate of removal. A similar amount of N was retained in the moss layer or  
494 soil (either in solution, sorbed to the solid phase, or immobilized in the microbial biomass)  
495 indicating that approximately 30-65% was lost by denitrification (as  $\text{NO}$ ,  $\text{N}_2\text{O}$  or  $\text{N}_2$ ),  
496 mass water flow, or translocated by roots out of the  $^{15}\text{N}$  addition area. Our results are  
497 consistent with short-term  $^{15}\text{N}$  recovery by vegetation in other non-riparian studies (e.g.  
498 grasslands; Nordbakken et al., 2003; Wilkinson et al., 2015). However, the high  
499 variability in  $^{15}\text{N}$  recovery between replicates, most likely due to inherent heterogeneity  
500 in riparian areas, made it difficult to identify any consistent spatial patterns in N uptake  
501 across the riparian transect (Williams et al., 2015). Additionally, only short-term fate of  
502 urea-N was studied and differences in mass flow under the vegetation (and therefore  $^{15}\text{N}$   
503 residence time) was not accounted for (Weaver et al., 2001).

504           The genes abundance of the different steps of the N cycle showed niche  
505 differentiation along the riparian transect. The *nifH* gene spatial distribution showed a  
506 strong link to areas with lower soil water content, bulk density and higher porosity and  
507  $\text{NH}_4^+$  concentrations, indicating the potential role of N fixation in zones 2 and 3 to  
508 accumulate  $\text{NH}_4^+$  in soil. This is consistent with these plant communities (e.g. *Calluna*-  
509 *Eriophorum* and *Sphagnum* species) being severely N limited (Leppanen et al., 2015). In  
510 contrast, AOA abundance followed the opposite trend than *nifH* gene, with the same  
511 factors explaining their distribution. Thus, we conclude that nitrogen fixation and  
512 nitrification are not coupled in the riparian wetland. This also implies that the archaea are  
513 the main microorganisms involved in nitrification over bacteria (Caffrey et al., 2007;

514 Erguder et al., 2009). Thus, despite AOA and AOB delivering the same function, the two  
515 communities live in distinct niches with different drivers. The low abundance of AOB is  
516 likely due to the low soil pH (4.05 – 4.90), that favour AOA (Leininger et al., 2006),  
517 while the drop in AOA abundance in distal zone could be related to the higher  
518 concentration of  $\text{NH}_4^+$  (Verhamme et al., 2011) or the change in soil water content.

519 Thus, the variation in ammonia oxidisers along the riparian transect will directly  
520 affect the rate of denitrification. The constant  $\text{NO}_3^-$  concentration along the transect,  
521 indicate that denitrification is occurring close to the river, which was confirmed by the  
522 potential denitrification rates, highly stimulated by C addition (glucose), and to a lesser  
523 extent by N (urea) in this area. It is well established that denitrification rates are usually  
524 enhanced by anoxic conditions, high  $\text{NO}_3^-$  availability and labile organic C (Weier et al.,  
525 1993). This is supported by the oligotrophic nature of the habitat, the high C-to-N ratio  
526 of the soil, and the recalcitrant nature of the plant litter produced by the vegetation (Witt  
527 and Setälä, 2010). Although the *Calluna* heath soil possessed high levels of DOC, this  
528 has previously been shown to be largely resistant to microbial attack due to its high  
529 aromatic content (Stutter et al., 2013). Interestingly,  $\text{N}_2\text{O}$  production was stimulated  
530 greatly in the *Juncus effusus* zone when labile C was added, however, there was not a  
531 cumulative effect after the addition of C and N together. The low concentrations of  $\text{NO}_3^-$   
532 in this zone also suggests that any  $\text{NO}_3^-$  produced could be lost to the river or is absorbed  
533 by plants. Overall, nitrification appears to be the rate limiting step in N cycling within the  
534 riparian wetland studied here.

535 With respect to the functional genes of the denitrifier community, none of the  
536 genes studied showed high abundance close to the river. Only the *nirS* gene displayed a  
537 higher abundance within zone 2, related to the increase in soil pH by less than a pH unit,  
538 highlighting the sensitivity of *nirS* gene abundance to pH (Liu et al., 2010). However, the

539 relatively higher abundance of *nirS* was not translated into higher N<sub>2</sub>O, although it should  
540 be noted that *nirS* and *nirK* code for nitrite reductase. The fungi, could also play a role in  
541 the denitrification as they possess *nirK* and *nirS* genes, which were not captured by the  
542 primers used. Some studies have indicated that N<sub>2</sub>O emissions from fungal communities  
543 can be significant as they lack the *nosZ* gene to reduce N<sub>2</sub>O to N<sub>2</sub>; their contribution in  
544 riparian areas remains uncertain and further work is needed to explore their role further  
545 (Ma et al., 2008; Seo and DeLaune, 2010).

546         It is difficult to conclude on the potential N<sub>2</sub>O emissions because the acetylene  
547 assay used in the study block the reduction of N<sub>2</sub>O into N<sub>2</sub>. The higher N<sub>2</sub>O emissions  
548 close to the river after C and N addition could then be reduced. However, the constant  
549 *nosZ* clade I gene abundance and the absence of *nosZ* clade II gene along the transect,  
550 might indicate that N<sub>2</sub>O is more likely to be emitted from the area close to the river, while  
551 the distal zone might be a sink for N<sub>2</sub>O. Therefore, from a management perspective,  
552 restricted access to grazing and OM amendments which are commonly used for wetland  
553 restoration to accelerate soil development and regulate soil moisture fluctuation, would  
554 be recommended to avoid future potential greenhouse gas emissions in wetlands under  
555 grazing regimes (Bruland et al., 2009).

556 .

## 557 **5. Conclusions**

558         In terms of preventing freshwater pollution, riparian areas represent one of the  
559 most valuable management tools for preventing excess nutrient loss from land to water.  
560 Most studies to date, however, have focused on N and P cycling and transformations in  
561 riparian soils adjacent to arable and intensively managed grasslands. Given the  
562 heterogeneous nature of land use in many catchments, and the trend towards modelling  
563 ecosystem services at the catchment scale, we need to gain a better understanding of

564 riparian N transformations across a variety of habitats and under different land use  
565 intensities. Our study in an extensively managed agricultural system clearly showed that  
566 changes in environmental factors such as breaks in vegetation or soil water saturation  
567 provide strong indicators of the relative importance of different biotic and abiotic  
568 processes involved in N cycling. However, our results also revealed hidden gradients in  
569 microbial community structure and N cycling gene abundance across the riparian strip.  
570 This reflects differences in key soil properties (e.g. organic matter content, redox) and  
571 also possibly the source of nutrients flowing through the soil (i.e. in hyporheic water flow  
572 versus lateral flow from upslope areas) and N<sub>2</sub>O fluxes. This type of spatial information  
573 can be used for more accurate mapping of ecosystem services at the catchment scale and  
574 the design of better livestock management systems (e.g. prevention of grazing in riparian  
575 areas to avoid N<sub>2</sub>O emissions). While we have provided novel insights into the dominant  
576 pathways for N removal in riparian zones, further work is required to investigate if  
577 seasonal patterns exist and how closely gene abundance is related to gene expression.

578

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587

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806 **Figure Legends**

807 **Fig. 1.** The Conwy catchment, North Wales, UK showing the location of the riparian  
808 sampling area and the major land cover classes.

809 **Fig. 2.** Location of sample points across the riparian area. Different colours indicate  
810 changes in vegetation. Zone 1 represents the area dominated by *Juncus effusus*,  
811 Zone 2 corresponds to the transitional area between the grasses and the heath, and  
812 Zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as  
813 the dominant species.

814 **Fig. 3.** Correlation bi-plot from the principal component analysis (PCA) on PLFA  
815 microbial groups (%) with respect to distance from the river ( $n = 4$ ). Zone 1  
816 represents the area dominated by *Juncus effusus* and is closest to the river (2 and 5  
817 m), zone 2 corresponds to the transitional area between the grasses and the heath  
818 (10 m), and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum*  
819 mosses as the dominant species and the farthest points from the river (15 and 25  
820 m). Correlation of PLFA microbial groups with the main axes are given by their  
821 specific names and distance from the river by cluster centroids (average score on  
822 each horizontal principal component (PC1) and vertical principal component (PC2)  
823 with standards errors). Circles represents sample points within the same zone.

824 **Fig. 4.** Total bacterial, archaeal and fungal gene copy numbers relative to distance from  
825 the river. Same lower case letters indicate no significant differences ( $P > 0.05$ ) with  
826 respect to distance from the river according to one-way ANOVA and the Tukey  
827 post-hoc test. Bars represent mean values ( $n = 4$  for 10, 15 and 25 m,  $n = 3$  for 2 m  
828 and  $n = 2$  for 5 m)  $\pm$  SEM. Distance from river corresponds to a change in the  
829 vegetation as shown in Figure 2.

830 **Fig. 5.** Recovery of  $^{15}\text{N}$  (% of total applied) from within the different fractions (shoots,  
831 roots, mosses and soil) represented by bars ( $n = 3$  except moss in zone 1 where  $n =$   
832 1). Zone 1 represents the area dominated by *Juncus effusus* and is closest to the  
833 river (5 m), zone 2 corresponds to the transitional area between the grasses and the  
834 heath (10 m) and zone 3 represents the heathland with *Calluna vulgaris* and  
835 *Sphagnum* mosses as the dominant species and the farthest points from the river (25  
836 m). Same lower case letters indicate no significant differences ( $P > 0.05$ ) with  
837 respect to the different fractions within each zone according to one-way ANOVA  
838 and Tukey post-hoc test.

839 **Fig. 6.** Cumulative  $\text{N}_2\text{O}$  emissions via denitrification in unamended soil (control) or after  
840 the application of labile C (glucose) and N (urea) either alone or in combination.  
841 Same lower case letters indicate no significant differences ( $P > 0.05$ ) with respect  
842 to distance from the river according to Welch's test and the Games-Howell post-  
843 hoc test. Same capital letters indicate no significant differences ( $P > 0.05$ ) between  
844 treatments for each distance from the river according to one-way ANOVA and  
845 Tukey post-hoc test. Bars represent mean values ( $n = 4$ )  $\pm$  SEM.

846 **Fig. 7.** Bacterial *amoA* (AOB), archaeal *amoA* (AOA), *nifH*, *nirS*, *nosK*, *nosZ* gene copy  
847 numbers relative to distance from the river. Same lower case letters indicate no  
848 significant differences ( $P > 0.05$ ) relative to distance from the river according to  
849 one-way ANOVA and the Tukey post-hoc test. Bars represent mean values ( $n = 4$   
850 for 10, 15 and 25 m,  $n = 3$  for 2 m and  $n = 2$  for 5 m)  $\pm$  SEM. Distance from river  
851 corresponds to a change in the vegetation as shown in Figure 2.

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855 **Research Highlights**

- 856 • Microbial community structure changed with distance from the river.
- 857 • *amoA* gene abundance increased towards the river while *nifH* decreased.
- 858 • N<sub>2</sub>O emissions rates were C limited but were greatest close to the river.
- 859 • Plant uptake of urea-<sup>15</sup>N was high across the riparian zone.
- 860 • The spatial pattern of N removal by riparian plants and microbes was different.

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863 **Table 1.** Soil physicochemical properties across the riparian transect. Different zones  
 864 indicate changes in vegetation community with zone 1 being closest to the river. Values  
 865 represent means ± SEM (*n* = 4). Same lower-case letters indicate no significant  
 866 differences (*P* > 0.05) with regard to distance from river according to One-way ANOVA  
 867 and Tukey or Games-Howell post-hoc test. Results are expressed on a soil dry weight  
 868 basis.

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Soil property	Zone 1		Zone 2	Zone 3	
	2 m	5 m	10 m	15 m	25 m
pH	4.18 ± 0.08 <sup>a</sup>	4.24 ± 0.05 <sup>a</sup>	4.90 ± 0.09 <sup>b</sup>	4.12 ± 0.02 <sup>a</sup>	4.05 ± 0.01 <sup>a</sup>
EC (µS cm <sup>-1</sup> )	23.4 ± 3.2 <sup>a</sup>	21.1 ± 2.0 <sup>a</sup>	11.6 ± 1.0 <sup>b</sup>	23.3 ± 2.4 <sup>a</sup>	26.3 ± 1.8 <sup>a</sup>
Bulk density (g cm <sup>-3</sup> )	0.31 ± 0.019 <sup>a</sup>	0.20 ± 0.026 <sup>a</sup>	0.09 ± 0.005 <sup>b</sup>	0.09 ± 0.004 <sup>b</sup>	0.09 ± 0.008 <sup>b</sup>
Total porosity (cm <sup>3</sup> cm <sup>-3</sup> )	0.78 ± 1.33 <sup>a</sup>	0.86 ± 1.88 <sup>ab</sup>	0.94 ± 0.36 <sup>b</sup>	0.94 ± 0.32 <sup>b</sup>	0.93 ± 0.54 <sup>b</sup>
Soil gravimetric water content (g kg <sup>-1</sup> soil)	659 ± 28 <sup>a</sup>	720 ± 5 <sup>a</sup>	793 ± 34 <sup>a</sup>	892 ± 2 <sup>b</sup>	899 ± 0.6 <sup>b</sup>
Organic matter (g kg <sup>-1</sup> soil)	364 ± 20 <sup>a</sup>	470 ± 12 <sup>b</sup>	542 ± 87 <sup>ab</sup>	953 ± 5 <sup>c</sup>	965 ± 4 <sup>c</sup>
NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> soil)	5.06 ± 0.95 <sup>a</sup>	4.75 ± 0.70 <sup>a</sup>	9.50 ± 1.56 <sup>ab</sup>	18.5 ± 1.94 <sup>b</sup>	16.7 ± 3.43 <sup>b</sup>
NO <sub>3</sub> -N (mg kg <sup>-1</sup> soil)	9.38 ± 0.92 <sup>a</sup>	12.6 ± 2.40 <sup>a</sup>	8.12 ± 3.09 <sup>a</sup>	10.5 ± 1.95 <sup>a</sup>	8.00 ± 0.91 <sup>a</sup>
Available P (mg kg <sup>-1</sup> soil)	5.82 ± 3.60 <sup>a</sup>	3.10 ± 1.11 <sup>a</sup>	5.99 ± 3.68 <sup>a</sup>	56.0 ± 10.1 <sup>b</sup>	50.5 ± 13.7 <sup>b</sup>
Total C (g kg <sup>-1</sup> soil)	215 ± 9 <sup>a</sup>	281 ± 8 <sup>b</sup>	330 ± 57 <sup>abc</sup>	576 ± 4 <sup>c</sup>	588 ± 17 <sup>c</sup>
Total N (g kg <sup>-1</sup> soil)	8.58 ± 0.61 <sup>a</sup>	12.0 ± 0.57 <sup>b</sup>	15.5 ± 2.58 <sup>ab</sup>	17.1 ± 0.11 <sup>c</sup>	15.7 ± 0.38 <sup>c</sup>
C-to-N ratio	25.3 ± 0.77 <sup>a</sup>	23.5 ± 0.92 <sup>ab</sup>	21.3 ± 0.53 <sup>b</sup>	33.8 ± 0.32 <sup>c</sup>	37.0 ± 1.96 <sup>c</sup>
Dissolved organic C (g kg <sup>-1</sup> soil)	0.24 ± 0.02 <sup>a</sup>	0.36 ± 0.02 <sup>bc</sup>	0.38 ± 0.07 <sup>ab</sup>	1.31 ± 0.18 <sup>cd</sup>	1.09 ± 0.14 <sup>cd</sup>
Total dissolved N (g kg <sup>-1</sup> soil)	0.04 ± 0.005 <sup>a</sup>	0.05 ± 0.005 <sup>a</sup>	0.06 ± 0.009 <sup>a</sup>	0.44 ± 0.29 <sup>a</sup>	0.11 ± 0.025 <sup>a</sup>
Microbial biomass PLFA (mmol kg <sup>-1</sup> soil)	1.12 ± 0.21 <sup>a</sup>	2.02 ± 0.27 <sup>a</sup>	3.83 ± 1.25 <sup>ab</sup>	7.58 ± 0.54 <sup>b</sup>	7.29 ± 1.70 <sup>b</sup>
AMOC (mg C-CO <sub>2</sub> kg <sup>-1</sup> soil h <sup>-1</sup> )	0.23 ± 0.04 <sup>a</sup>	0.41 ± 0.06 <sup>ab</sup>	0.61 ± 0.16 <sup>ab</sup>	0.92 ± 0.14 <sup>b</sup>	0.98 ± 0.20 <sup>b</sup>
AMN (mg kg <sup>-1</sup> soil)	69.0 ± 12.2 <sup>a</sup>	116 ± 13.7 <sup>a</sup>	104 ± 15.2 <sup>a</sup>	96.0 ± 8.27 <sup>a</sup>	97.8 ± 30.0 <sup>a</sup>

870 Electrical conductivity (EC). Phospholipid Fatty Acid Analysis (PLFA). Anaerobically mineralization  
 871 organic carbon (AMOC). Anaerobically mineralization nitrogen (AMN).

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882 **Table 2.** Spearman's rank correlation coefficients and *P*-values between soil  
883 physicochemical properties and abundance of functional genes (gene copies  $\mu\text{g}^{-1}$  DNA).  
884 Significant correlations are shown in bold.

Functional genes	Bacterial <i>16SrRNA</i>	Archaeal <i>16SrRNA</i>	Fungal <i>ITS</i>	<i>nifH</i>	Bacterial <i>amoA</i>	Archaeal <i>amoA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>
pH	<b>0.478</b>	0.131	0.071	-0.055	0.066	<b>0.614</b>	0.275	<b>0.515</b>	<b>0.495</b>
<i>p</i> -value	<b>0.033</b>	0.583	0.788	0.833	0.801	<b>0.009</b>	0.286	<b>0.034</b>	<b>0.043</b>
EC	<b>-0.450</b>	-0.128	-0.018	-0.151	-0.170	-0.471	<b>-0.522</b>	<b>-0.522</b>	<b>-0.627</b>
<i>p</i> -value	<b>0.047</b>	0.590	0.944	0.563	0.513	0.057	<b>0.031</b>	<b>0.031</b>	<b>0.007</b>
Bulk density	-0.040	<b>-0.568</b>	0.440	<b>-0.699</b>	0.419	<b>0.723</b>	-0.450	0.368	0.184
<i>p</i> -value	0.867	<b>0.009</b>	0.077	<b>0.002</b>	0.094	<b>0.001</b>	0.070	0.146	0.480
Total porosity	0.043	<b>0.562</b>	<b>-0.427</b>	<b>0.704</b>	-0.414	<b>-0.726</b>	0.454	-0.365	-0.168
<i>p</i> -value	0.856	<b>0.010</b>	<b>0.087</b>	<b>0.002</b>	0.098	<b>0.001</b>	0.067	0.150	0.519
Soil water content	-0.057	0.378	-0.249	<b>0.592</b>	-0.215	<b>-0.907</b>	0.215	-0.407	-0.316
<i>p</i> -value	0.810	0.101	0.335	<b>0.012</b>	0.408	<b>0.000</b>	0.408	0.105	0.216
Organic matter	-0.171	0.338	-0.218	<b>0.597</b>	-0.244	<b>-0.907</b>	0.261	-0.421	-0.360
<i>p</i> -value	0.471	0.144	0.400	<b>0.011</b>	0.345	<b>0.000</b>	0.311	0.093	0.155
NH <sub>4</sub> <sup>+</sup> -N	0.135	0.427	-0.108	<b>0.582</b>	-0.195	<b>-0.669</b>	0.297	-0.387	-0.333
<i>p</i> -value	0.571	0.060	0.680	<b>0.014</b>	0.453	<b>0.003</b>	0.247	0.125	0.191
NO <sub>3</sub> -N	-0.236	-0.237	0.400	-0.173	-0.147	0.071	-0.387	-0.240	-0.184
<i>p</i> -value	0.317	0.314	0.112	0.507	0.573	0.786	0.124	0.352	0.480
Available P	0.103	<b>0.485</b>	-0.081	<b>0.457</b>	0.129	<b>-0.618</b>	0.116	-0.166	-0.218
<i>p</i> -value	0.665	<b>0.030</b>	0.757	<b>0.065</b>	0.622	<b>0.008</b>	0.656	0.525	0.400
Total C	-0.161	0.407	-0.294	<b>0.577</b>	-0.258	<b>-0.869</b>	0.253	-0.412	-0.440
<i>p</i> -value	0.497	0.075	0.252	<b>0.015</b>	0.318	<b>0.000</b>	0.328	0.100	0.077
Total N	-0.023	0.358	-0.007	<b>0.795</b>	-0.300	<b>-0.632</b>	<b>0.490</b>	-0.317	-0.105
<i>p</i> -value	0.925	0.121	0.978	<b>0.000</b>	0.241	<b>0.006</b>	<b>0.046</b>	0.216	0.687
Dissolved organic C	-0.029	0.343	-0.106	<b>0.580</b>	-0.201	<b>-0.674</b>	0.200	-0.361	-0.439
<i>p</i> -value	0.902	0.139	0.687	<b>0.015</b>	0.439	<b>0.003</b>	0.442	0.155	0.078
Total dissolved N	-0.062	0.236	0.058	<b>0.544</b>	-0.217	<b>-0.610</b>	0.201	-0.374	-0.341
<i>p</i> -value	0.796	0.317	0.826	<b>0.024</b>	0.403	<b>0.009</b>	0.439	0.139	0.181
Microbial biomass PLFA	-0.026	0.276	-0.044	<b>0.639</b>	-0.229	<b>-0.806</b>	0.256	-0.373	-0.203

<i>p</i> -value	0.912	0.238	0.866	<b>0.006</b>	0.376	<b>0.000</b>	0.321	0.140	0.434
AMOC	-0.229	-0.263	0.314	0.256	-0.294	-0.181	-0.009	-0.276	0.108
<i>p</i> -value	0.331	0.263	0.220	0.321	0.252	0.486	0.974	0.283	0.680
AMN	-0.033	0.057	0.171	<b>0.611</b>	-0.181	<b>-0.544</b>	0.316	-0.222	-0.049
<i>p</i> -value	0.890	0.810	0.513	<b>0.009</b>	0.486	<b>0.024</b>	0.216	0.392	0.852

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885 Electrical conductivity (EC). Phospholipid Fatty Acid Analysis (PLFA). Anaerobically mineralizable  
886 organic carbon (AMOC).

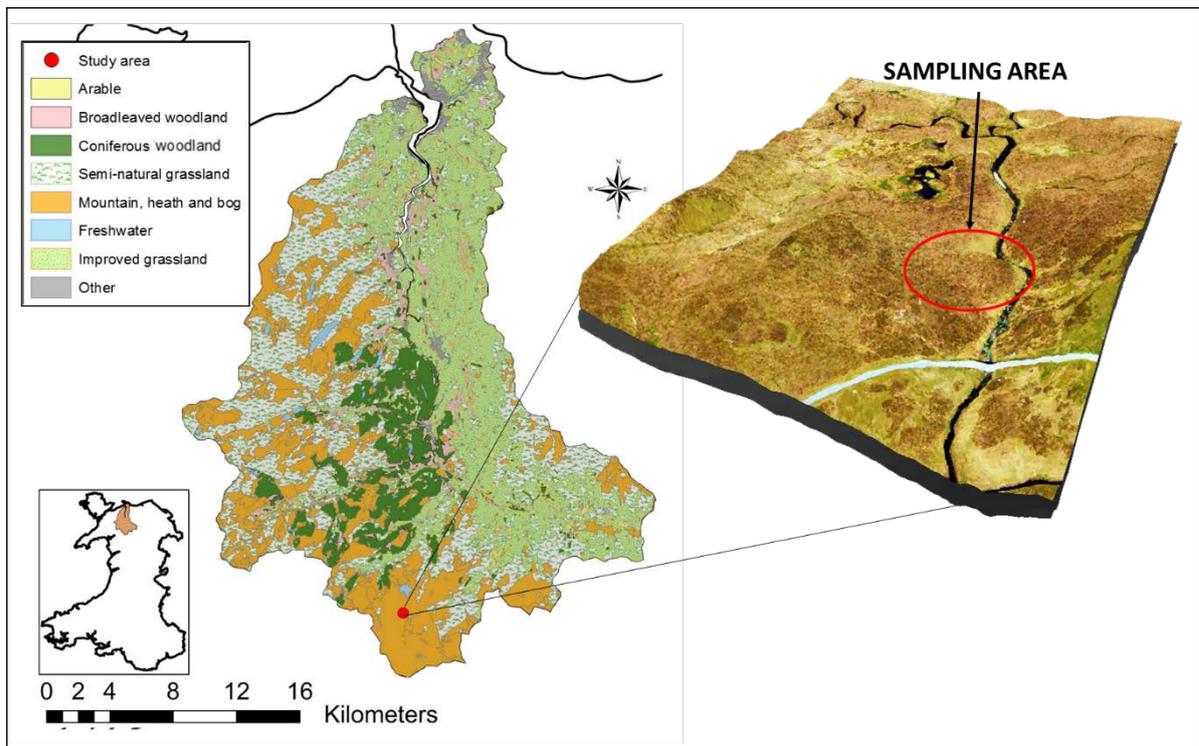
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888 **Table 3.** Spearman's rank correlation coefficients and *P*-values between soil  
 889 physicochemical properties and N<sub>2</sub>O emission (mg N kg<sup>-1</sup> h<sup>-1</sup>) in unamended soil (control)  
 890 or after the addition of labile C and N.

Soil property	N <sub>2</sub> O emissions (Control)	N <sub>2</sub> O emissions (C addition)	N <sub>2</sub> O emissions (N addition)	N <sub>2</sub> O emissions (C and N addition)
Water content	0.24	-0.80	-0.71	-0.81
<i>p</i> -value	0.316	<0.001	<0.001	<0.001
Bulk density	-0.33	0.73	0.70	0.79
<i>p</i> -value	0.152	<0.001	0.001	<0.001
Total nitrogen	0.19	-0.89	-0.65	-0.74
<i>p</i> -value	0.431	<0.001	0.002	<0.001
Total porosity	0.33	-0.74	-0.69	-0.80
<i>p</i> -value	0.152	<0.001	0.001	<0.001
AMOC	0.31	-0.86	-0.70	0.82
<i>p</i> -value	0.179	<0.001	0.001	<0.001

891 Anaerobically mineralizable organic carbon (AMOC).

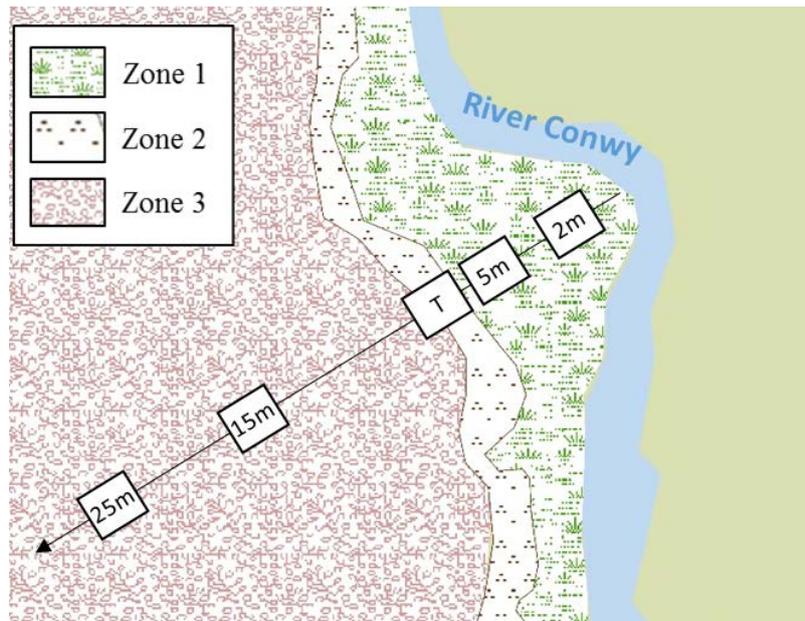
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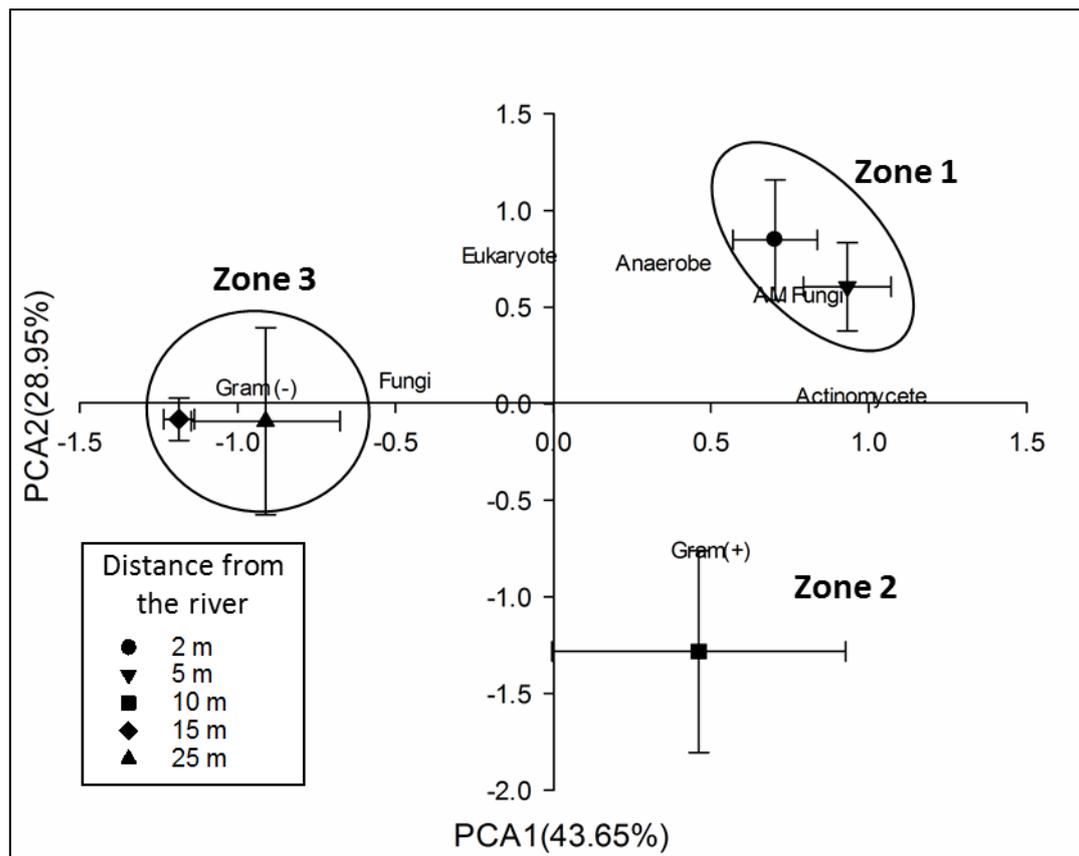
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894 **Fig. 1.** The Conwy catchment, North Wales, UK showing the location of the riparian  
 895 sampling area and the major land cover classes.

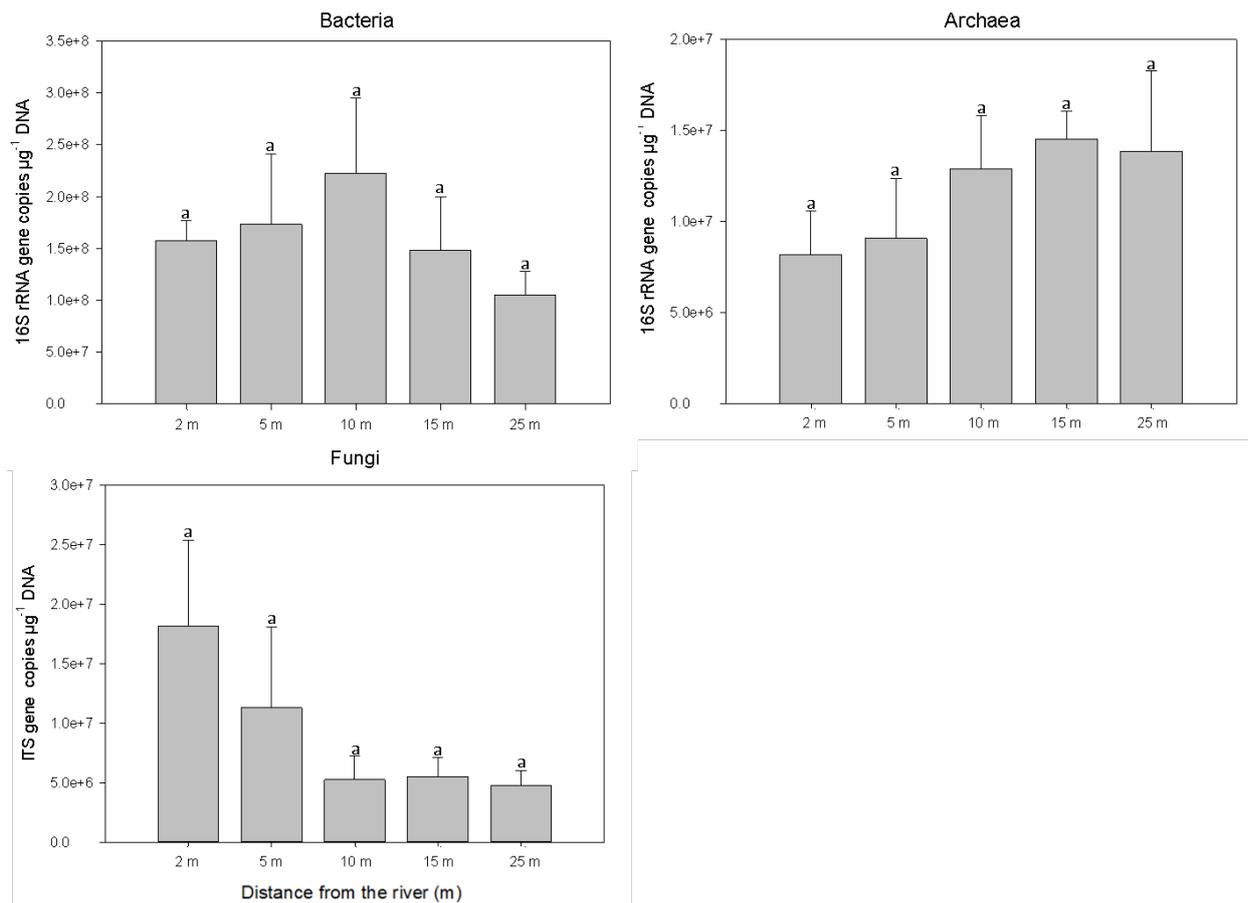
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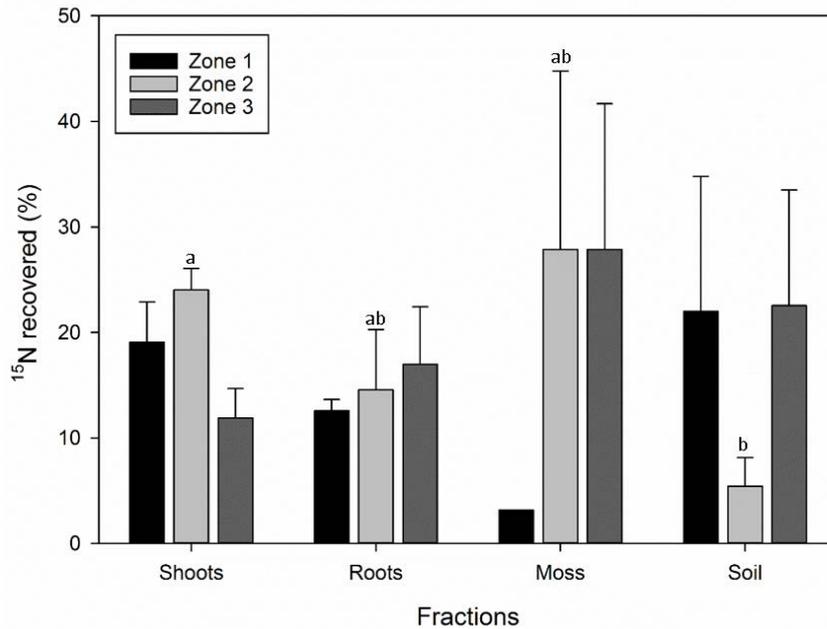
**Fig. 2.** Location of sample points across the riparian area. Different colours indicate changes in vegetation. Zone 1 represents the area dominated by *Juncus effusus*, Zone 2 corresponds to the transitional area between the grasses and the heath, and Zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species.



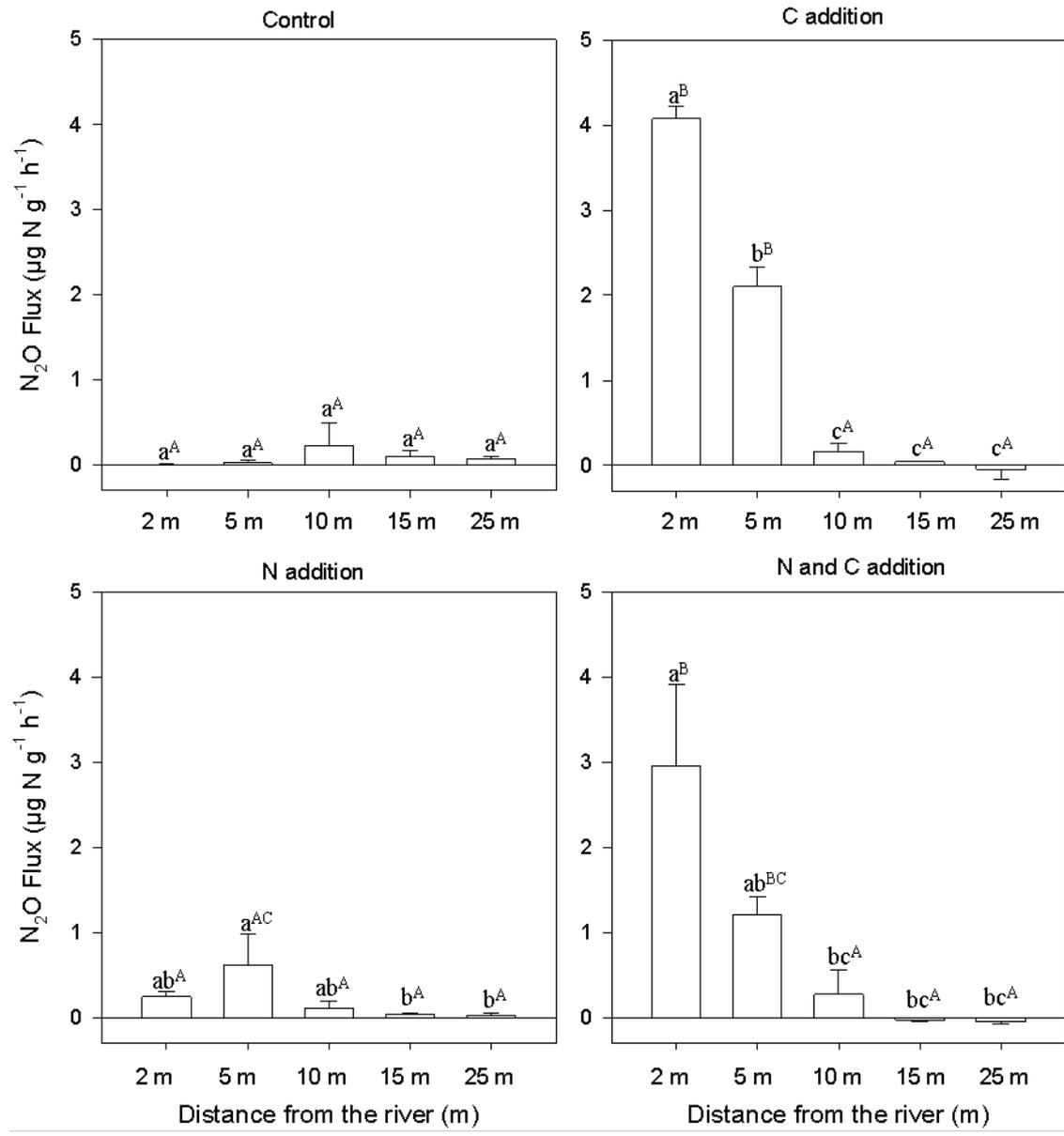
**Fig. 3.** Correlation bi-plot from the principal component analysis (PCA) on PLFA microbial groups (%) with respect to distance from the river ( $n = 4$ ). Zone 1 represents the area dominated by *Juncus effusus* and is closest to the river (2 and 5 m), zone 2 corresponds to the transitional area between the grasses and the heath (10 m), and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species and the farthest points from the river (15 and 25 m). Correlation of PLFA microbial groups with the main axes are given by their specific names and distance from the river by cluster centroids (average score on each horizontal principal component (PC1) and vertical principal component (PC2) with standards errors). Circles represents sample points within the same zone.



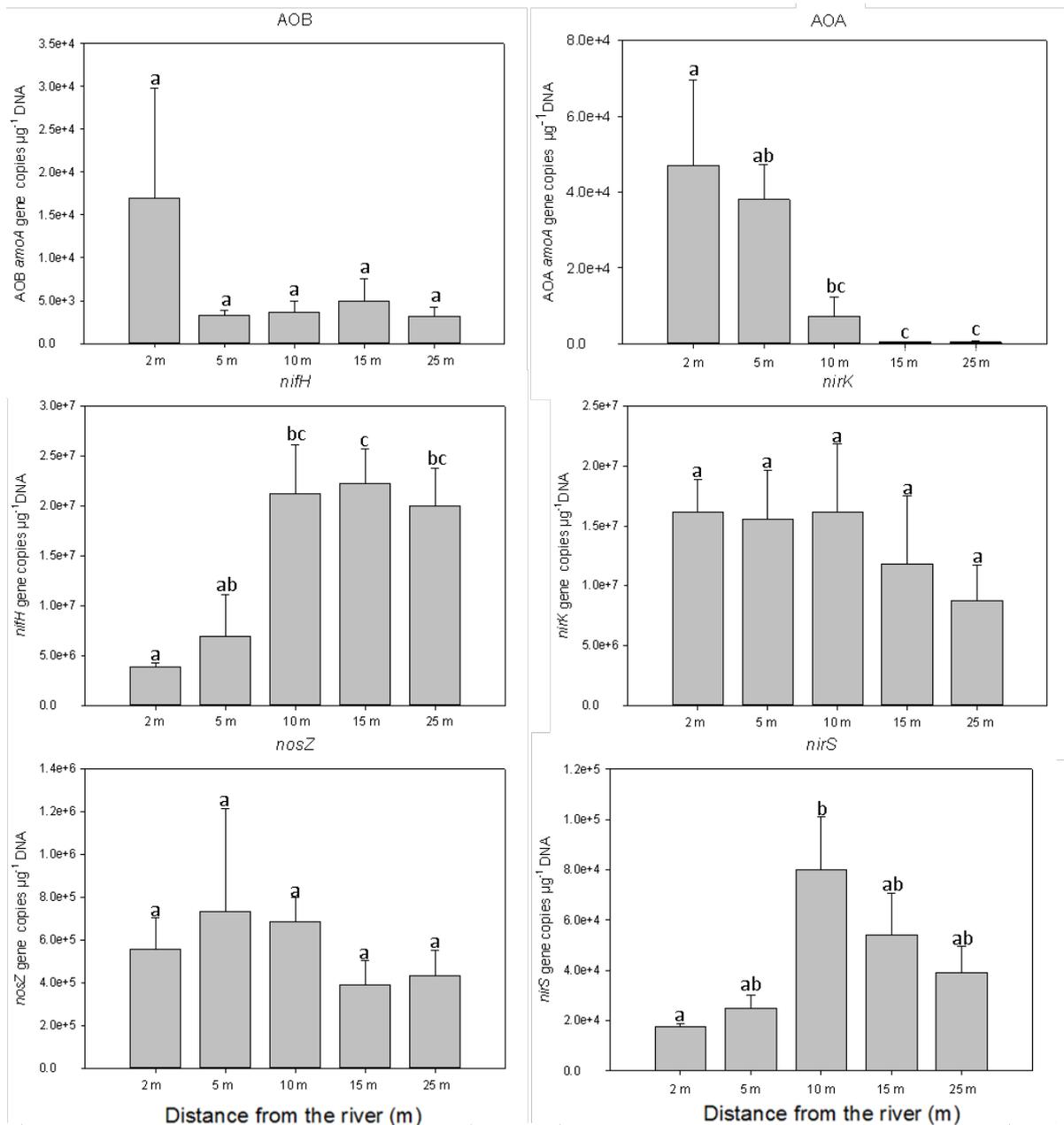
**Fig. 4.** Total bacterial, archaeal and fungal gene copy numbers relative to distance from the river. Same lower case letters indicate no significant differences ( $P > 0.05$ ) with respect to distance from the river according to one-way ANOVA and the Tukey post-hoc test. Bars represent mean values ( $n = 4$  for 10, 15 and 25 m,  $n = 3$  for 2 m and  $n = 2$  for 5 m)  $\pm$  SEM. Distance from river corresponds to a change in the vegetation as shown in Figure 2.



**Fig. 5.** Recovery of  $^{15}\text{N}$  (% of total applied) from within the different fractions (shoots, roots, mosses and soil) represented by bars ( $n = 3$  except moss in zone 1 where  $n = 1$ ). Zone 1 represents the area dominated by *Juncus effusus* and is closest to the river (5 m), zone 2 corresponds to the transitional area between the grasses and the heath (10 m) and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species and the farthest points from the river (25 m). Same lower case letters or the lack of it indicate no significant differences ( $P > 0.05$ ) with respect to the different fractions within each zone according to one-way ANOVA and the Tukey post-hoc test.



**Fig. 6.** Cumulative N<sub>2</sub>O emissions via denitrification in unamended soil (control) or after the application of labile C (glucose) and N (urea) either alone or in combination. Same lower case letters indicate no significant differences ( $P > 0.05$ ) with respect to distance from the river according to Welch's test and the Games-Howell post-hoc test. Same capital letters indicate no significant differences ( $P > 0.05$ ) between treatments for each distance from the river according to one-way ANOVA and Tukey post-hoc test. Bars represent mean values ( $n = 4$ )  $\pm$  SEM.



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2 **Fig. 7.** Bacterial *amoA* (AOB), archaeal *amoA* (AOA), *nifH*, *nirS*, *nosK*, *nosZ* gene copy  
 3 numbers relative to distance from the river. Same lower case letters indicate no significant  
 4 differences ( $P > 0.05$ ) relative to distance from the river according to one-way ANOVA  
 5 and the Tukey post-hoc test. Bars represent mean values ( $n = 4$  for 10, 15 and 25 m,  $n =$   
 6  $3$  for 2 m and  $n = 2$  for 5 m)  $\pm$  SEM. Distance from river corresponds to a change in the  
 7 vegetation as shown in Figure 2.

8 **Spatial zoning of microbial function and plant-soil nitrogen dynamics across a**  
 9 **riparian area**

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11 Laura L. de Sosa<sup>a,\*</sup>, Helen C. Glanville<sup>a,b</sup>, Miles R. Marshall<sup>c</sup>, A. Prysor Williams<sup>a</sup>, Maïder  
12 Abadie<sup>d</sup>, Ian M. Clark<sup>d</sup>, Aimeric Blaud<sup>d</sup>, Davey L. Jones<sup>a</sup>

13 <sup>a</sup> *School of Environment, Natural Resources & Geography, Bangor University, Deiniol*  
14 *Road, Bangor, Gwynedd, LL57 2UW, United Kingdom*

15 <sup>b</sup> *School of Geography, Geology and the Environment, Keele University, Keele,*  
16 *Staffordshire, ST5 5BG, United Kingdom*

17 <sup>c</sup> *Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Rd, Bangor,*  
18 *Gwynedd, LL57 2UW, United Kingdom*

19 <sup>d</sup> *Department of Sustainable Agriculture Sciences, Rothamsted Research, West Common,*  
20 *Harpenden, Hertfordshire, AL5 2JQ, United Kingdom*

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22

23

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28 \* Corresponding author. School of Environment, Natural Resources & Geography,  
29 Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, United Kingdom.

30 *E-mail address:* [afs411@bangor.ac.uk](mailto:afs411@bangor.ac.uk) (L.L. de Sosa).

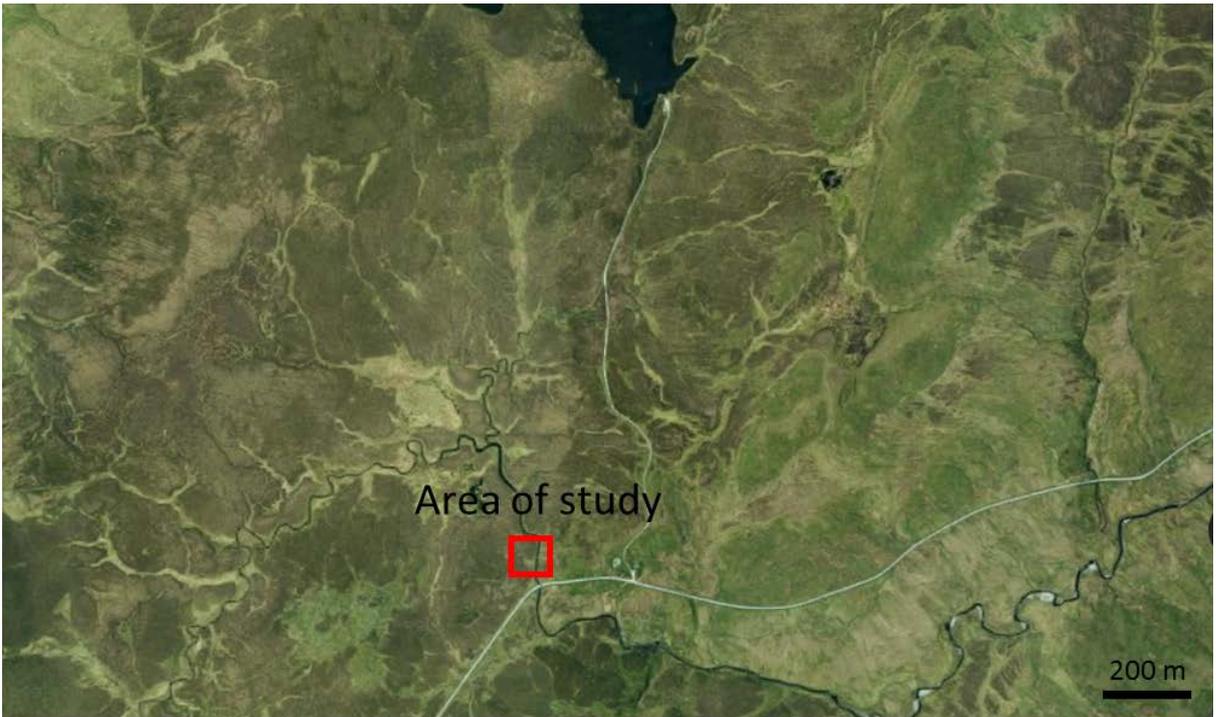
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34 **Supplementary on-line information**

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37 **Fig. S1.** Aerial photography of the area of study.

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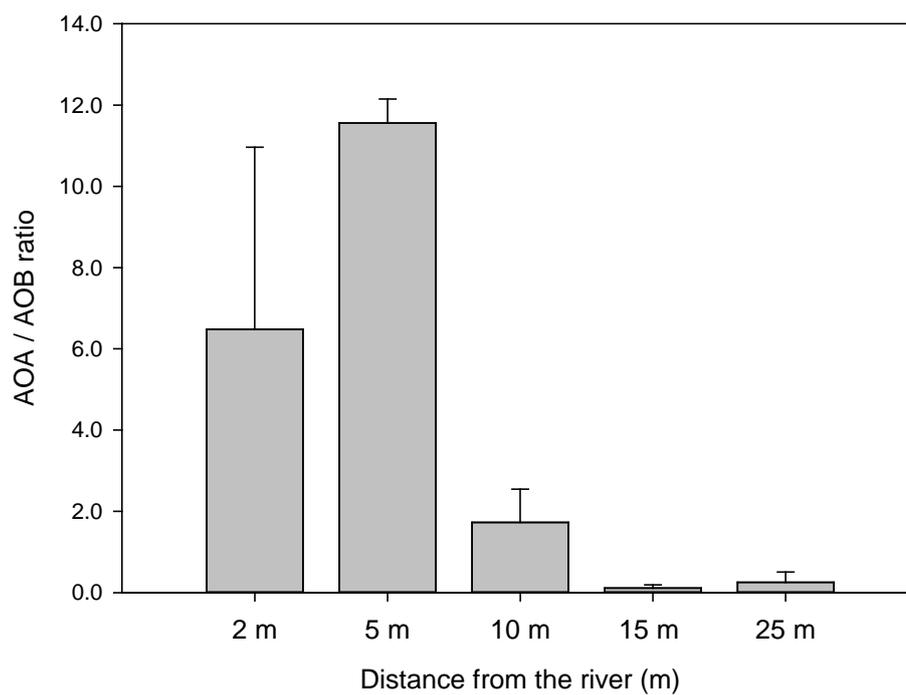
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44 **Fig. S2.** Detailed photographs of vegetation in the area of study.

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48 **Fig. S3.** Ratios of AOA to AOB *amoA* copy numbers relative to distance from the  
49 river. Bars represent mean values ( $n = 4$  for 2, 10, 15 and 25 m and  $n = 2$  for 5 m)  
50  $\pm$  SEM. Distance from river corresponds to a change in the vegetation as shown in  
51 Figure 3.

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66 **Table S1.** PLFA biomarkers used for taxonomic microbial groups

Microbial group category	PLFA specific fatty acids			
AM Fungi	16:1 w5c			
Saprophytic Fungi	18:2 w6c			
Gram Negative	10:0 2OH	14:0 2OH	18:1 w6c	21:1 w8c
	10:0 3OH	16:1 w9c	18:0 cyclo w6c	21:1 w6c
	12:1 w8c	16:1 w7c	18:1 w3c	21:1 w5c
	12:1 w5c	16:1 w6c	19:1 w9c	21:1 w4c
	13:1 w5c	16:1 w4c	19:1 w8c	21:1 w3c
	13:1 w4c	16:1 w3c	18:1 w5c	22:1 w9c
	13:1 w3c	17:1 w9c	19:1 w6c	22:1 w8c
	12:0 2OH	17:1 w8c	19:0 cyclo w9c	22:1 w6c
	14:1 w9c	17:1 w7c	19:0 cyclo w7c	22:1 w5c
	14:1 w8c	17:1 w6c	9:1 w17c	22:1 w3c
	14:1 w7c	17:1 w5c	20:1 w9c	22:0 cyclo w6c
	14:1 w5c	17:1 w4c	20:1 w8c	24:1 w9c
	15:1 w9c	17:1 w3c	20:1 w6c	24:1 w7c
	15:1 w8c	16:0 2OH	19:0 cyclo w6c	11:0 iso 3OH
	15:1 w7c	17:0 cyclo w7c	20:1 w4c	14:0 iso 3OH
15:1 w6c	18:1 w8c	20:0 cyclo w6c		
15:1 w5c	18:1 w7c	21:1 w9c		
Methanotroph	16:1 w8c			
Eukaryote	15:4 w3c	19:3 w3c	22:5 w6c	23:3 w3c
	15:3 w3c	20:4 w6c	22:6 w3c	23:1 w5c
	16:4 w3c	20:5 w3c	22:4 w6c	23:1 w4c
	16:3 w6c	20:3 w6c	22:5 w3c	24:4 w6c
	18:3 w6c	20:2 w6c	22:2 w6c	24:3 w6c
	19:4 w6c	21:3 w6c	23:4 w6c	24:3 w3c
	19:3 w6c	21:3 w3c	23:3 w6c	24:1 w3c
Gram Positive	11:0 iso	14:0 iso	16:0 iso	17:1 anteiso w7c
	11:0 anteiso	14:0 anteiso	16:0 anteiso	19:0 iso
	12:0 iso	15:1 iso w9c	17:1 iso w9c	19:0 anteiso
	12:0 anteiso	15:1 iso w6c	17:0 iso	20:0 iso
	13:0 iso	15:1 anteiso w9c	17:0 anteiso	22:0 iso
	13:0 anteiso	15:0 iso	18:0 iso	
Anaerobe	14:1 iso w7c	15:0 anteiso	17:1 anteiso w9c	
	12:0 DMA	15:0 DMA	16:1 w5c DMA	18:1 w7c DMA
	13:0 DMA	16:2 DMA	16:0 DMA	18:1 w5c DMA
	14:1 w7c	17:0 DMA	18:2 DMA	18:0 DMA
	DMA	16:1 w9c DMA	18:1 w9c DMA	
	14:0 DMA	16:1 w7c DMA		
Actinomycetes	15:0 iso DMA			
	16:0 10-methyl	18:1 w7c 10-methyl	22:0 10- methyl	
	17:1 w7c 10-methyl	18:0 10-methyl	20:0 10- methyl	
	17:0 10-methyl	19:1 w7c 10-methyl		

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68 **Table S2.** List of the primers used to target each community.

Target gene	Primer	Sequence 5'-3'	References
Bacterial <i>16SrRNA</i>	341F	CCT AYG GGR BGC ASC AG	Glarling et al. (2015)
	806R	GGA CTA CNN GGG TAT CTA AT	
Archaeal <i>16SrRNA</i>	Parch519F	CAG CMG CCG CGG TAA	Øvreaset al. (1997)
	Arch1060R	GGC CAT GCA CCW CCT CTC	Reysenbach and Pace, (1995)
Fungal <i>ITS</i>	ITS1f	TCC GTA GGT GAA CCT GCG G	Gardes and Bruns (1993); Vilgalys and Hester (1990)
	5.8s	CGC TGC GTT CTT CAT CG	
<i>nifH</i>	PolF	TGC GAY CCS AAR GCB GAC TC	Poly et al. (2001)
	PolR	ATS GCC ATC ATY TCR CCG GA	
<i>amoA</i> Bacteria	amoA-1F	GGG GTT TCT ACT GGT GGT	Rotthauwe et al. (1997)
	amoA-2R	CCC CTC KGS AAA GCC TTC TTC	
<i>amoA</i> Archaea	Arch-amoAF	STA ATG GTC TGG CTT AGA CG	Francis et al. (2005)
	Arch-amoAR	GCG GCC ATC CAT CTG TAT GT	
<i>nirK</i>	nirK876F	ATY GGC GGV CAY GGC GA	Henry et al. (2004)
	nirK1040R	GCC TCG ATC AGR TTR TGG TT	
<i>nirS</i>	cd3aF	GTS AAC GTS AAG GAR ACS GG	Throback et al. (2004)
	R3cdR	GAS TTC GGR TGS GTC TTG A	
<i>nosZ</i>	nosZ1F	CGC RAC GGC AAS AAG GTS MSS GT	Henry et al. (2006)
	nosZ1R	CAK RTG CAK SGC RTG GCA GAA	
<i>nosZII</i>	nosZ-II-F	CTI GGI CCI YTK CAY AC	Jones et. al (2013)
	nosZ-II-R	GCI GAR CAR AAI TCB GTR C	

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77 **Table S3.** Phospholipid fatty acid (PLFA) ratios of main microbial groups. Values  
 78 represent means  $\pm$  SEM ( $n = 4$ ). Same lower case letters indicate no significant differences  
 79 ( $P > 0.05$ ) with respect to distance from the river according to one-way ANOVA and the  
 80 Tukey post-hoc test.

PLFA ratio	Zone 1		Zone 2		Zone 3	
	2 m	5 m	10 m	15 m	25 m	
Fungi/Bacteria	0.07 $\pm$ 0.01 <sup>a</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	0.04 $\pm$ 0.004 <sup>b</sup>	0.03 $\pm$ 0.005 <sup>b</sup>	0.04 $\pm$ 0.006 <sup>b</sup>	
Predator/Prey	0.03 $\pm$ 0.003 <sup>a</sup>	0.03 $\pm$ 0.001 <sup>a</sup>	0.02 $\pm$ 0.004 <sup>a</sup>	0.03 $\pm$ 0.004 <sup>a</sup>	0.03 $\pm$ 0.005 <sup>a</sup>	
Gram +/Gram -	0.76 $\pm$ 0.03 <sup>ab</sup>	0.83 $\pm$ 0.04 <sup>b</sup>	0.88 $\pm$ 0.10 <sup>b</sup>	0.56 $\pm$ 0.01 <sup>c</sup>	0.61 $\pm$ 0.03 <sup>ac</sup>	
Saturated/Unsaturated	1.01 $\pm$ 0.09 <sup>a</sup>	1.05 $\pm$ 0.10 <sup>a</sup>	1.38 $\pm$ 0.26 <sup>a</sup>	0.74 $\pm$ 0.04 <sup>a</sup>	0.88 $\pm$ 0.18 <sup>a</sup>	
Mono/Poly	13.5 $\pm$ 1.18 <sup>a</sup>	14.4 $\pm$ 2.68 <sup>a</sup>	15.9 $\pm$ 1.76 <sup>a</sup>	17.6 $\pm$ 1.50 <sup>a</sup>	16.6 $\pm$ 2.44 <sup>a</sup>	
16w/16 cyclo	3.31 $\pm$ 0.19 <sup>a</sup>	3.89 $\pm$ 0.23 <sup>a</sup>	4.11 $\pm$ 0.53 <sup>a</sup>	9.65 $\pm$ 0.54 <sup>b</sup>	9.20 $\pm$ 0.69 <sup>b</sup>	
18w/19 cyclo	0.70 0.07 <sup>a</sup>	0.64 0.04 <sup>a</sup>	0.85 0.11 <sup>a</sup>	1.93 0.08 <sup>b</sup>	2.06 0.25 <sup>b</sup>	

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