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Local functioning, landscape structuring:

drivers of soil microbial community structure and function in peatlands

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Abstract

Agricultural peatlands are essential for a myriad of ecosystem functions and play an important role in the global carbon (C) cycle through C sequestration. Management of these agricultural peatlands takes place at different spatial scales, ranging from local to landscape management, and drivers of soil microbial community structure and function may be scale-dependent. Effective management for an optimal biogeochemical functioning thus requires knowledge of the drivers on soil microbial community structure and functioning, as well as the spatial scales upon which they are influenced. During two field campaigns, we examined the importance of different drivers (i.e. soil characteristics, nutrient management, vegetation composition) at two spatial scales (local vs landscape) for, respectively, the soil microbial community structure (determined by PLFA) and soil microbial community functional capacity (as assessed by CLPP) in agricultural peatlands. First, we show by an analysis of PLFA profiles that the total

27 microbial biomass changes with soil moisture and relative C:P nutrient availability. Secondly,
28 we showed that soil communities are controlled by a distinct set of drivers at the local, as
29 opposed to landscape, scale. Community structure was found to be markedly different between
30 areas, in contrast to community function which showed high variability within areas. We further
31 found that microbial structure appears to be controlled more at a landscape scale by nutrient-
32 related variables, whereas microbial functional capacity is driven locally through plant
33 community feedbacks. Optimal management strategies within such peatlands should therefore
34 consider the scale-dependent action of soil microbial community drivers, for example by first
35 optimizing microbial structure at the landscape scale by targeted areal management, and then
36 optimizing soil microbial function by local vegetation management.

37

38 **Introduction**

39 Peatlands play an important role in Earth's biogeochemical cycles by storing about an estimated
40 third of all terrestrial carbon (C) (Turetsky et al. 2002, Turunen et al. 2002). In Europe, the
41 majority of peatlands is in use as agricultural land (Joosten and Clarke 2002). Despite their
42 potential to sequester C, agricultural peatlands typically act as significant C sources. Worldwide
43 drainage of such peatlands has increased the rates of peat oxidation and hence microbial
44 decomposition, causing high rates of C losses and greenhouse gas emissions (Drösler et al.
45 2008). However, due to the large C sequestration potential of agricultural peatlands, they could
46 play an important role in efforts to increase soil C storage, such as the recently launched '4 per
47 1000 initiative', which seeks to increase C storage in agricultural soils with 4% per year (Le
48 Foll 2015).

49

50 Current peatland management influences microbe-mediated biogeochemical functions, for
51 example by maintaining waterlogged conditions to prevent microbial peat oxidation and

52 thereby reduce peat subsidence and CO₂ emissions (Kløve *et al.*, 2017). Restoration of peat
53 ditches often seeks to optimize nutrient removal and reduce eutrophication, both of which have
54 links to the microbial processes of nitrogen (N) and phosphorus (P) conversion. Microbial
55 activities are clearly critical to the success of peatland management strategies, for instance for
56 C storage, yet management practises rarely consider potential impacts on soil-borne microbial
57 communities. With future climate change pressures in mind, the management of ecosystems for
58 minimal microbial mediated CH₄ and N₂O emission is expected to become ever more important
59 (Taft *et al.* 2017).

60

61 Traditionally regarded as random noise, spatial variability in soil microbial communities is now
62 widely acknowledged (Ettema and Wardle 2002), and it displays consistent and informative
63 patterns at different spatial scales (O'Brien *et al.* 2016). With mounting evidence for scale-
64 dependent ecological processes acting on microbial communities, the need for examining
65 multiple spatial scales to understand the patterns in soil microbial communities has become
66 apparent (Martiny *et al.* 2011). This has led to the study and discovery of clear examples of
67 small scale (cm to m) patterns (e.g. Franklin and Mills 2003), as well as large continental
68 (Waldrop *et al.* 2017) and global biogeographic patterns in soil microbial communities
69 (Nemergut *et al.* 2011). These scales of study are not always in line with the scales at which the
70 management of such ecosystems takes place. For agricultural peatlands, the most obvious scale
71 of management is that of the field level, with local farmers carrying out customary management
72 practices such as fertilization, grazing and mowing. Another relevant scale is that of the
73 landscape level at which spatial planning and water level management occurs. Furthermore, the
74 different components of the ecosystem may also be organized at different spatial scales
75 themselves (Yergeau *et al.* 2010). To adequately steer management towards optimization of

76 microbial communities, there is a need to match the spatial scale of land management and the
77 study of spatial microbial community patterns.

78

79 While identification of patterns in soil microbial composition is in itself relevant, there is a clear
80 need to go beyond pattern description and towards identification of the underlying drivers of
81 community structure and functioning (Martiny et al. 2011, Hanson et al. 2012). Soil microbial
82 community structure and functioning are often assumed to be driven by the same factors
83 (O'Brien et al. 2016). This would imply that management aimed at an optimal microbial
84 structure will also result in the desired functioning of the ecosystem. Alternatively, soil
85 microbes are often considered to have high functional redundancy (Strickland et al. 2009); and
86 therefore drivers of soil microbial community structure may have minimal effect on soil
87 microbial functioning. This perspective would imply that management practices designed to
88 control only the drivers of community function would be sufficient to achieve the desired
89 ecosystem functions.

90

91 Soil microbial community structure has been shown to be controlled by a wide spectrum of
92 drivers: soil pH and C:N ratio (Lauber et al. 2008, Fierer et al. 2009, 2012, Kuramae et al. 2012,
93 Zhang et al. 2013, Ramirez et al. 2014), vegetation (O'Donnell et al. 2001, de Vries et al. 2012),
94 external nutrient load (O'Donnell et al. 2001, O'Brien et al. 2016), and soil moisture (Brockett
95 et al. 2012). Although drivers of soil microbial community function have not been examined in
96 as much detail, but it has been shown that soil microbial community functions are also
97 controlled by soil moisture (Brockett et al. 2012), C:N ratio (Kuramae et al. 2014), and external
98 nutrient load and pH (Wakelin et al. 2013). These different drivers of community structure and
99 functioning differ with the spatial scale of examination. As land management is carried out at
100 scales orders of magnitude larger than those experienced by microbes directly, it remains to be

101 tested if and how microbial communities respond to changes in these drivers. If soil microbial
102 community structure and functioning are influenced by scale-dependent drivers, information on
103 the scale-dependency of these microbial community drivers could be useful for informing
104 management designed to improve peatland functioning.

105

106 In this study, we assessed the impacts of several drivers of soil microbial structuring and
107 functional capacity at local and landscape scales in agricultural peatlands. We combine data
108 from two sampling campaigns across agricultural peatlands in The Netherlands (Fig. 1a, 1b),
109 one that examined drivers of soil microbial community structure, as determined by
110 phospholipid fatty acid (PLFA) analysis, and one that examined drivers of soil microbial
111 community functional capacity, as estimated by community level physiological profiling
112 (CLPP). These patchwork agricultural landscapes are highly heterogeneous, making them
113 effective model systems for examining the effects of multiple environmental drivers on soil
114 microbial communities (Vasseur et al. 2013). Comparison of samples within and between
115 different polder areas made it possible to analyse drivers at two different spatial scales: local
116 scale (sites within a sampled area) and landscape scale (differences between sampled polder
117 areas).

118

119 **Methods**

120

121 *Study sites and design*

122 The field sites used in this study are situated in a peat area in the West of The Netherlands (Fig.
123 1a, Table 1) and comprise nine ± 200 ha peatlands (Referred to as ‘sampling areas’, Fig. 1b,
124 each comprising between 18 and 24 sampling sites). All areas are characterized by a mixture of
125 intensive and extensively managed peatlands intersected by ditches, resulting in a mosaic of

126 land uses. In the summer of 2013, a total of three agricultural peatland areas were sampled, and
127 another six areas were sampled in the subsequent summer of 2014. In each area, between 18
128 and 24 transects were laid out on field margins (referred to as 'sampling sites' Fig. 1c), as such
129 edges account for 96% of the total vegetation species richness of a field (Kleijn et al. 2001).
130 Each transect had a total length of 100 meter, where the vegetation was surveyed for the sloping
131 part of each transect up to the waterside (Fig. 1d). Vegetation abundance was assessed using
132 Tansley abundance classes (Tansley 1946), which were subsequently converted into abundance
133 percentages (Table S1). To analyse soil physical-chemical properties and microbial community
134 structure and functional capacity, five soil samples (10 cm deep) were taken in each transect,
135 20 meters apart from each other and 20 cm from the waterside (Fig. 1d). Soil samples were
136 mixed per transect after removal of the vegetation layer, sieved (2 mm mesh) and stored at -
137 80°C as one composite sample.

138

139 *Phospholipid fatty acid (PLFA) analyses*

140 Soil microbial community structure was determined by analysing Phospholipid Fatty Acids
141 (PLFA) extracted from soil samples taken in the areas that were sampled in summer 2013 (three
142 areas, 63 samples). PLFAs were extracted from 4 grams of soil per composite sample using an
143 adapted protocol, following White *et al.* (1979) and Frostegård and Bååth (1996). Lipid
144 fractionation took place over prepacked Bond Elut SI solid phase extraction columns, after
145 which lipid extracts were identified by gas chromatography (GC-FID, 7890A, Agilent
146 technologies, Delaware, USA). The (relative) abundance of of fungi, Gram positive (G⁺) and
147 Gram negative (G⁻) bacteria was characterized by the use of specific indicator PLFA
148 biomarkers: fungi (18:2 ω 6), G⁺ bacteria (i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0) and G⁻
149 bacteria (16:1 ω 7, cy17:0 and 18:1 ω 7). Total bacterial biomass was determined by taking the

150 sum of all bacterial biomarkers, including the general biomarker 15:0. Abundance of each
151 PLFA biomarker was expressed as nmol PLFA g⁻¹ dry weight of soil.

152

153 *Community level physiological profiling (CLPP)*

154 Functional diversity of the soil microbial community was determined in soil samples
155 originating from the areas that were sampled in summer 2014 (six areas, 144 samples) by the
156 use of Biolog EcoPlates (Biolog, Hayward, CA, USA). These 96-well plates contain three
157 replicate sets of 31 ecologically relevant C substrates, along with a tetrazolium redox dye
158 (Insam, 1997). Microbial use of these substrates is reflected by colour change in each of the
159 wells, as the redox dye is reduced to tetrazolium violet (Pohland & Owen, 2009). Eco-plate
160 wells were inoculated with diluted soil slurries (150 µl), obtained by mixing 0.5 gram of soil
161 with 49.5 mL of milli-Q water, shaken (200 rpm) for 30 minutes on an orbital shaker, and 10⁻⁴
162 diluted by serial dilution. Three technical replicates were included for each of the 144 soil
163 samples. Eco-plates were incubated in the dark at 25°C, and colour development was recorded
164 as optical density (OD₅₉₀, OD₇₅₀) at the start and after 24, 48, 72, 96, 168 and 192 hours, on a
165 Biotek Synergy HT plate reader (Biotek Instruments, Winooski, United States).

166

167 Conceptually, the function of Ecoplate-substrate utilisation through time consists of four
168 distinct phases (Fig. S1a). The substrate utilization function captures the signals of community
169 respiration, but also that of the substrate consumed for community growth. For our purposes,
170 we were interested only in the respiration of the originally sampled community. To remove the
171 signal of reproduction from the data, we used a modified method of Brouns *et al.* (2016). The
172 rationale behind this method is that by removing the exponential-growth signal from the
173 exponential phase of the substrate-use function, only the substrate use of the initial community
174 remains. The exponential phase is characterized by plentiful substrate where growth of

175 organisms is not limited by its availability. By fitting a log-linear regression to the extracted
176 exponential phase (Fig. S1b), we determined the initial community substrate use (y-intercept).
177 In contrast to the existing methodology, we determined the phase of true exponential growth
178 from the second derivative of a polygonal curve fit. By finding the inflection point, the point
179 where the second derivative changes from positive to negative, the convex, true exponential,
180 part of the curve is determined. We also accounted for the possible existence of a lag phase by
181 removing non-positive values (i.e. zeroes). We calculated the classical Average Well Colour
182 Development (AWCD; Garland and Mills 1991) and diversity metrics of substrate utilization
183 (e.g. Gomez et al. 2006), substrate richness, the exponent of the Shannon diversity and Pielou's
184 evenness of the substrate utilization.

185

186 *Soil chemical analyses*

187 Soil pH was measured after shaking a soil-water (1:2.5 w/v) suspension on an orbital shaker at
188 200 rpm for 2 hours. Total C, N and P analyses were performed on oven-dried (60°C, 96 h) and
189 ground (1.0 mm, Retsch SM 100, Haan, Germany) soil samples. Total C and N were determined
190 using an Elemental Analyser (Thermo Electron, Milan, Italy). Total P was determined
191 according to the method of Murphy and Riley (1962). Soil samples were ashed at 550 °C for
192 30 minutes, after which P was re-suspended by digestion with 2.5% (w/v) acid persulphate in
193 an autoclave (30 minutes at 121°C). Total P was measured colorimetrically, on a continuous
194 flow analyser (SEAL analytical, Abcoude, The Netherlands). Soil moisture was determined as
195 the percentage weight loss upon oven drying.

196

197 *Cartographic information*

198 Soil typological information, yearly fertilizer use and land management were extracted from
199 geographical maps (Alterra, PAWN; Natuur op Kaart, Kadaster 2013/14, SNL, IPO 2013/14)

200 using ArcGIS 10.1. With this information, we determined fertilizer use and N and P loadings
201 per hectare. In determining artificial and organic fertilizer (manure), we assumed that farmers
202 used the maximum amount of admissible fertilizer based on national legislation. Fields with
203 specific nutrient management schemes, such as areas with natural grassland management
204 generally use less artificial fertilizer due to a resting period where no fertilizer can be applied
205 or due to legal restrictions on artificial fertilizer use. Also, manure application may be
206 constrained due to the resting period or further limited to solid manure application for certain
207 types of nature management. In designated natural grassland sites, neither artificial nor organic
208 fertilizer application is allowed. We estimated inorganic and organic N and P loadings per
209 hectare (ha) per year for fields in each polder (Table 2, Table S2).

210

211 *Data analysis*

212 All analyses were performed in R version 3.2.1 using the vegan, KernSmooth, MASS, PCNM,
213 packfor and VennDiagram packages. In this study, we use two separate datasets on soil
214 microbial communities. One dataset contained data on soil microbial community structure
215 (PLFA data), and another dataset contained data on soil microbial community functional
216 capabilities (CLPP data). The PLFA data consisted of three areas containing 22, 18 and 23 sites,
217 each. The CLPP data encompassed 6 distinct areas with 24 sites each. First, we examined
218 general soil properties, biomass and PLFA and CLPP patterns in these datasets. We determined
219 descriptive soil properties (soil C,N,P content, pH and moisture) and tested how microbial
220 biomass changed along environmental gradients using generalized linear models with a gamma
221 distribution and log link function to deal with deviations from normality. These models were
222 run for the different proportions of biomass as calculated from the PLFA data as dependent
223 variables and included all soil geochemical, as well as all land management-related variables
224 and the polder area identity as explanatory variables. Second, to assess general patterns and

225 clustering in polder areas we used a principal component analysis (PCA). We tested the
226 importance of general drivers and the existence of polder level differences further using
227 distance-based redundancy analysis models (dbRDA; Legendre and Anderson, 1999), where
228 microbial community variation (in composition or functional capacity) between sites was
229 expressed as an Odum's percentage difference distance. Thus, large distances indicate very
230 different sites and small distances indicate comparable sites in terms of community structure or
231 functioning. We defined two spatial scale levels for this analysis, the local field level within
232 polders and between polder areas at the regional level. At both scale levels, we carried out a
233 variation partitioning analysis (Peres-Neto et al. 2006) using dbRDA. Prior to variation
234 partitioning, a dbRDA analysis on the full data set was carried out. Next, all models were
235 subjected to a forward selection procedure prior to variation partitioning (Blanchet et al. 2008).
236 We subsequently assessed the importance of underlying variables in shaping the microbial
237 community structure and functional capacity at the two scale levels by examining the explained
238 variation (R^2_{adj}) of the selected variables in isolation.

239

240 *Local scale: model definition*

241 Differences in community composition or functional capacity at the local scale may result from
242 differences in environmental quality between field edges. To analyse patterns at the local level,
243 we applied the approach described by Declerck et al. (2011). Briefly, dummy-coded polder
244 identifiers delineate the different study areas. These polder identifiers were used as covariates
245 in the analysis to control for large-scale patterns in the data. By controlling for the polder
246 identity, we could effectively study within polder patterns in community structure and function
247 for multiple polders simultaneously. We distinguished four explanatory models at the polder
248 level: a soil characteristics model (SOIL), a nutrient management model (NUT), a vegetation
249 composition model (VEG) and a spatial model (SPACE).

250

251 The SPACE model was composed of Moran Eigenvector Map (MEM) variables that explain
252 the spatial autocorrelation between sites in the landscape based on geographical distances
253 (PCNM: Dray et al. 2006). By using staggered matrices of MEM eigenvectors (Declerck et al.
254 2011, Legendre et al. 2013), we described spatial autocorrelation among sites within polders.
255 We only selected the eigenvectors with positive spatial correlations for analyses. The SPACE
256 model represents small scale spatial patterning in community variation, large scale patterning
257 already being excluded through the use of polder identity as a covariate. Our SOIL model
258 consisted of variables describing the quality of the soil (pH, C, N and P content, C:N, C:N and
259 N:P ratio, moisture), morphometric characteristics (bank angle, bank width) and soil typology.
260 Our NUT model consisted of loadings of organic and inorganic N and P applied to the field
261 along with dummy coded variables of the occurring nutrient management schemes (Table S4).
262 Our VEG model consisted of a staggered matrix of the principal axes of a principal coordinate
263 analysis (PCoA) per polder. Because many axes of a PCoA explained little to no variation, we
264 selected for relevant axes based on a broken stick model of explained variation, with all axes
265 before the break point being selected. The resulting axes were arranged into a staggered matrix
266 (Legendre et al. 2013) with the goal of only representing within polder differences. All models
267 were subjected to forward selection based on a double stopping criterion (R^2_{adj} and $\alpha > 0.05$) and
268 tested for significance using 99,999 permutations constrained within polder identity levels.

269

270 *Regional scale: model definition*

271 Differences in community composition or functioning at the landscape scale may result from
272 differences in environmental quality between polders. Environmental gradients existing at the
273 spatial grain of the landscape may be markedly different from those at the field level. Hence,
274 an examination of these gradients irrespective of the variation explained within polders is

275 appropriate. To this end, we used an approach where we first constructed a statistical model
276 explaining community variation by dummy-coded polder identity variables (Polder model). By
277 taking the predicted values of this polder model, we obtained a matrix of community variation
278 present between polder landscapes only. We used this matrix as our response matrix in
279 subsequent analyses of drivers of community variation between polders, allowing us to make
280 models that only explain community variation encompassed by the polder model. Here, we
281 constructed three explanatory models at the level of the landscape: a soil characteristics model
282 (SOIL), a nutrient loading model (NUT) and a vegetation composition model (VEG). For the
283 sake of interpretation, the explained variation of the models was rescaled to the total community
284 variation captured by the polder model.

285

286 Our SOIL and NUT models consisted of the same variables as those used at the local scale. Our
287 VEG model was created by transformation of an Odum's percentage dissimilarity matrix of the
288 vegetation composition of all field edges within the respective data set by means of a principal
289 coordinate analysis (PCoA). For the model explaining community variation, we made use of
290 the resulting PCoA axes. This approach differs from the one used at the local scale in that we
291 did not use PCoA axes per polder, but rather examined variation across all polders. As for the
292 local model, we selected for the relevant PCoA axes based on a broken stick model of explained
293 variation, with all axes before the break point being selected. The uniquely explaining part of
294 the variation of the polder model, the part not explained by environmental drivers, may be
295 interpreted as spatial patterning at the landscape level that is not directly related to the measured
296 environment. A formal permutation test is not viable with the limited number of different
297 polders. Hence, forward selection was carried out using the increase in R^2_{adj} as the only
298 criterion. Additionally, when models were found to be non-significant in explaining patterns in
299 the full data, irrespective of spatial scale, we disregarded the model in this analysis.

300 **Results**

301

302 *Soil chemical properties and microbial biomass patterns*

303 We examined soil chemical properties of our two data sets by calculating mean and spread of
304 the soil chemical properties for all sample areas (Table 2 and Table 3). The sample areas used
305 in the assessment of soil community structure analyses, showed a wide range in nutrient
306 content (Table 2) as well as in soil moisture levels across the different sample areas. Areas
307 also showed a wide range in microbial biomass (Table 2), which persisted across different
308 groups (Fig S2). The microbial biomass increased with decreasing relative soil P-content
309 (measured as molar C:P ratio and N:P) and was positively correlated to soil moisture levels
310 (Fig. 2, Table S3). The range of soil properties (nutrient content and soil moisture) was even
311 greater for the samples examined by CLPP, though average soil chemical properties were
312 within the same general range (Table 3). Substrate utilization was largely comparable between
313 areas, though varied considerably within areas (Table 3, Fig. S4 S5).

314 *Drivers of soil microbial community structure at different spatial scales*

315 Soil microbial community structure was examined by PLFA fingerprinting. In a first
316 examination of PCA results (Fig. 3), we found clear differences between the different polder
317 areas examined. A dbRDA of the PLFA data revealed that 19.8% of the community variation
318 could be explained by differences between polders (Fig. 5a; Table S4c) and a mere 4.0% could
319 be explained within polders (Fig 5b; Table S4a). Nonetheless, we were able to identify
320 consistent, significant gradients explaining community structure (Table S4). At the local scale
321 (Fig. 5b), only the NUT model proved to explain a significant portion of community variation
322 ($R^2_{\text{adj}}=4.0\%$, $P<0.01$). This leaves large parts of the total variation explained at the level of the
323 full dataset unaccounted for (Table S5). A part of this community variation was found to be
324 explained at the landscape scale instead (Fig. 5a) by means of the SOIL, NUT and VEG models.

325 Only 2.9% (ns) of the variation was unique to the polder model, and not captured by one of the
326 other models (Fig. 5a, Table S4a). The SOIL model was the most important explaining
327 environmental component ($R^2_{\text{adj}}=16.1\%$), encompassing large parts of the explained variation
328 of the NUT (2.7%+1.1%=3.8%) and VEG model (3.0%+1.1%=4.1%).

329

330 We ranked variables underlying the main drivers identified in the variation partitioning in terms
331 of their importance (Table 4). At the landscape scale, PLFA structuring responded most
332 strongly to nutrient-related variables, the soil P content (10.3%), inorganic N fertilization
333 (3.1%), soil N content (1.7%), organic P (1.0%) and N fertilization (0.6%). In addition to
334 nutrient-related parameters, soil type (7.8%), the presence of nature management schemes
335 (2.6%), agri-environmental schemes (0.2%), and the resident vegetation composition (4.6%)
336 were shown to be important variables in explaining landscape level community structure. At
337 the local scale, less of the variation in PLFA data was explained, with organic P fertilization
338 being the most pronounced driver (5.7%) of microbial community structure.

339

340 *Drivers of microbial community functional capacity at different spatial scales*

341 Community level physiological profiling (CLPP) was used as a proxy for the functional
342 capacity of the microbial community. In a first examination of PCA results (Fig. 4), we found
343 a strong overlap between sites of the different polder areas under examination. This was also
344 reflected in RDA analyses of the data, with only 5.0% of the total variation in community
345 functional capacity being explained by the polder model (Fig. 5c). Despite this small part of the
346 variation being explained, we did find that part of the CLPP variation between landscapes was
347 associated with soil characteristics ($R^2_{\text{adj}}=2.8\%$) and vegetation ($R^2_{\text{adj}}=2.2\%$) (Fig. 5c). Nutrient
348 management was found to be non-significant in explaining patterns in the full dataset (Table
349 1), and it therefore did not explain any of the variation encompassed by differences between

350 polders. At a local scale (Fig. 5d), we could explain a larger part of the variation (13.9%), which
351 was attributed to the vegetation composition model ($R^2_{\text{adj}}=8.7\%$, $P<0.001$) and spatial patterns
352 in community functional capacity ($R^2_{\text{adj}}=8.8\%$, $P<0.001$).

353

354 We identified the primary driving variables related to soil microbial community functional
355 capacity (Table 4). At a the landscape scale, soil pH (1.1%) and soil type (0.9%) and soil P
356 ratios (soil N:P: 0.7% and soil C:P: 0.6%) were found to be most explaining for the variation in
357 functional capacity. The local scale was explained by the vegetation community and a spatial
358 MEM model based on geographical distance between field edges. The latter showed that most
359 patterns were described by the highest order MEM variable (7.7%), indicative of a relatively
360 coarse spatial patterning of community functioning.

361

362 *Comparing community structure and functional capacity*

363 Comparing the two datasets, the two analyses of community variation yielded highly disparate
364 results with respect to the scale at which different environmental factors could explain variation
365 in the data (Fig. 5). Community structure data (PLFA) was associated with environmental
366 factors between different polders, *i.e.* at a large landscape scale (Fig. 5, Table S4). In contrast,
367 functional data was poorly explained at this scale; rather environmental variation within polders
368 offered the greatest level of explanatory power (Fig. 5). Despite the difference in total explained
369 variation, at the landscape scale the general partitioning and relative weight of the drivers was
370 comparable for both PLFA structure and CLPP (Fig. 5, Table S4). Both microbial community
371 properties were most explained by the SOIL model with a small contribution of the variance
372 being explained by VEG. Moreover, variation was highly collinear between the different
373 models. On a local scale, patterns were markedly different between community structure and
374 functional capacity.

375

376 **Discussion**

377 Understanding the drivers of soil microbial processes at relevant scales can help to improve
378 management of agricultural peatlands to protect and improve desired ecosystem functioning.
379 Through our analyses, we have examined the driving forces of microbial community structure
380 and functioning in field margins along agricultural banks at two different scale levels; within
381 polders (local) and between polders (landscape). We found local and landscape scale drivers to
382 be distinct at different scale levels. The underlying variables were found to be largely different
383 as well. This implies that the spatial scale of soil microbial studies is important when talking
384 about driving forces of soil microbial community structure and functioning, enforcing the idea
385 that the scale of soil management and the scale of study of soil microbial structure and
386 functioning need to be well aligned.

387

388 *Local functioning, landscape structuring*

389 While somewhat anecdotal due to the separate collection of the datasets, we showed that soil
390 microbial community structure (PLFA) was more strongly regulated at the landscape scale,
391 while functional capacity (CLPP) was more strongly driven at the local scale. Explained
392 variation, while not being exceptionally high (15-20%), was comparable to other studies using
393 similar multivariate community analysis approaches (Van der Gucht et al. 2007, Sayer et al.
394 2017). Future studies could consider integrated methods that address both structure and
395 functioning conjointly (e.g. ^{13}C PLFA, Yao et al. 2015). The inclusion of additional
396 environmental drivers, such as specific fractions of bio-available nutrient pools, would
397 potentially have increased the amount of explained variation. Across polder regions, e.g. at the
398 landscape scale, the results indicate a driving role for soil characteristics, with vegetation being
399 largely collinear with soil characteristics (for similar findings see: Kuramae et al. 2010). We

400 therefore conclude that, with respect to soil microbial structure, differences in vegetation and
401 nutrient management between polders are well reflected in the soil characteristics. Local
402 microbial structure could only be led back to the applied nutrient management of the field and
403 explained little variation. In contrast, variation in community functional capacity could be
404 explained better by vegetation composition and spatial patterns at the local scale, with both
405 explaining distinctly different parts of the community variation. The overlap in drivers at the
406 landscape scale is likely due to the fact that the studied areas vary in land-use, land-history and
407 management, which leads to landscape-scale vegetation and nutrient availability patterns that
408 leave clear imprints in the soil. Locally, the small-scale heterogeneity of fields becomes more
409 important in driving the specific microbial function. This mismatch in scale between structure
410 and function has been described previously for specific microbes and their functions (Veraart
411 et al. 2017).

412

413 *Drivers of soil microbial community structuring and functional capacity*

414 Drivers of community variation may differ strongly with scale (Yergeau et al. 2010, Prober et
415 al. 2015), and our analyses support this premise. At both scale levels, community structure was
416 driven by nutrient management. The latter result is in agreement with previous research
417 (O'Donnell et al. 2001, O'Brien et al. 2016) that has shown the importance of fertilization
418 regimes for soil microbial communities. In turn, the supply and manner in which nutrients are
419 added can have direct consequences for ecosystem functions such as nutrient retention and plant
420 uptake (Heijboer et al. 2016). We, however, did find clear differences in underlying drivers of
421 nutrient management of the within and between polder scales, with organic P loading and
422 inorganic N loading being most important. This highlights the importance of identifying
423 underlying drivers (Martiny et al. 2011). By focussing on a single scale level, important drivers

424 may be overlooked and incorrect conclusions may arise, potentially leading to mismanagement
425 of the agricultural landscape.

426 Our conclusions regarding landscape scale patterns are complicated by the lack of extensive
427 replication at the landscape level, making formal testing of the drivers encapsulated within the
428 polder model problematic. While we acknowledge these limitations within our study, our
429 results are strengthened by the strong significant patterns found in tests of the entire data set
430 (Table S4). As large parts of the total variation that can be explained by our models remain
431 unexplained at the local scale (e.g. Fig. 2b), it is reasonable to assume that this variation may
432 be explained at the landscape scale.

433

434 A surprising similarity in soil characteristic drivers of soil microbial community structure and
435 functioning can be found for nutrient-related drivers (soil N:P ratio, soil P and N content).
436 Specifically, soil N content was found as the only variable that was important in determining
437 landscape scale community structure, as well as the community functional capacity.
438 Additionally, for community structure, specifically P-related processes were important drivers
439 at a local (organic P fertilization) and at a landscape scale (soil P content, organic P
440 fertilization). Soil nutrient content and the relative P availability compared to other nutrients
441 were also primary drivers of microbial biomass. In existing literature, little attention has been
442 paid to the effects of P on peatland microbial communities and functioning (Lin et al. 2014,
443 Veraart et al. 2015). Our results suggest that these effects of P enrichment on peatland microbial
444 communities deserve additional consideration.

445

446 The relevance of the resident vegetation community for local microbial functional capacity, but
447 not local microbial structure, is a noteworthy result. This could be caused by the study design
448 in which we compare different polder areas with slightly different plant communities. An

449 ecological explanation for this may be found in the stimulating role of plant presence and
450 diversity on the function of soil microbes by (e.g. Zak *et al.*, 2003). Furthermore, a well-
451 developed, species-rich riparian zone will influence water and nutrient retention (Hefting *et al.*
452 2005) and thereby microbial functioning (Korol *et al.* 2016). This development of a riparian
453 zone depends strongly on local disturbance by mowing and cattle grazing. We did not directly
454 quantify these factors, although they should in part be represented in the nutrient and land
455 management schemes. However, within these schemes, there is room for variation in grazing
456 and mowing regimes at the digression of the land manager. As land managers tend to own
457 different nearby fields within a landscape, this variation in mowing a grazing is likely to be
458 spatially structured. Our results, where vegetation and spatial structure explain local functional
459 capacity, may thus be (partially) explained by these unmeasured differences in management
460 regimes.

461

462 We found evidence for spatial patterns that could not be explained by any of the measured
463 environmental drivers at the level of the local functional capacity (uniquely explained variation
464 of the SPACE model), which may represent a possible signal of dispersal limitation (Dray *et al.*
465 2006). While dispersal-limitation has been shown to be plausible within microbial
466 communities (Evans *et al.* 2017, Langenheder *et al.* 2017), it is rarely a significant driver of
467 microbial community structure (Martiny *et al.* 2011, O'Brien *et al.* 2016). Hence, our observed
468 spatial patterns are likely to be caused by spatially-structured environmental variables (e.g. light
469 climate, soil redox conditions, readily available nutrient fractions, available substrates) that
470 were not taken into account in this study (Martiny *et al.* 2006, Yao *et al.* 2011).

471

472 *Management of soil microbial communities in peatlands: an integrative approach*

473 Our results suggest that microbial function is regulated by multiple different drivers that are
474 distinct from those driving soil microbial structure, and that these drivers act at different spatial
475 scales. This complicates the task of managing agricultural peatlands for desired ecological
476 functioning. The traditional view maintains that environmental drivers influence community
477 structure and that this structure in turn influences community functioning (Allison and Martiny
478 2008). However, this paradigm has been proven to be insufficient to explain microbial
479 functional patterns in nature (Strickland et al. 2009, Weedon et al. 2017). Microbial functions
480 have been shown to change independently of microbial community structure (Tian et al. 2016,
481 Weedon et al. 2017) and respond to different variables than structure (Boeddinghaus et al.
482 2015). However, disregarding community structure entirely and solely focussing on functioning
483 is also clearly inappropriate, as microbial community structure serves as a constraint on the
484 realized functioning of the community and the ecosystem as a whole (Pérez-Valera et al. 2015,
485 Heijboer et al. 2016).

486

487 We argue that for effective management of desired functioning to optimize the different societal
488 benefits obtained from the landscape, both soil microbial structure and functioning need to be
489 considered. Based on our study, environmental quality changes relevant for soil microbial
490 functional capacity were most pronounced at the local scale. As local environmental quality
491 shifts, this may lead to a direct shift in realized functioning away from the desired function (Fig.
492 3, horizontal axis). However, the magnitude of this shift may be limited by the community
493 structure, which constraints the extent of the shift in function (e.g. compare Fig. 3, central-right
494 and bottom-right, respectively unconstrained vs constrained situation). Changes in
495 environmental drivers governing structure (Fig. 3, vertical axis) were primarily found to
496 manifest themselves at the level of the landscape within the context of this study. A change in
497 environment at the landscape level may hamper realization of the desired function by

498 constraining the realised function negatively as well (e.g Fig 3, top-left). Hence, a thorough
499 understanding of the community structure and its potential to facilitate the desired function is
500 an imperative first step in soil microbial management, followed by optimization of the
501 conditions directly driving required soil microbial functioning. Throughout this process, the
502 spatial scale at which microbial structure and functioning responds to these changes needs to
503 be taken into account. Landscape measures, such as water level fluctuations and spatial
504 planning set the constraints for the potential functioning (i.e. structure), and once this stage has
505 been set, local management options such as mowing and fertilization regimes are decisive in
506 determining if the desired functioning can be achieved.

507

508 **Concluding remarks**

509 Our study showed that soil microbial communities of agricultural peatlands are driven by
510 different factors at distinct, management-relevant spatial scales. Furthermore, our study
511 provides a first indication that soil community structure and function do not necessarily respond
512 to the same factors, or at the same spatial scales. We argue that it is important to take both these
513 soil microbial community characteristics (structure and function) into account for management
514 of these important ecosystems. Based on this study, we suggest optimizing management of
515 microbial ecosystem functioning in peatlands by first focussing on landscape restoration,
516 followed by suitable local scale management optimization. This is directly relation to recent
517 initiatives such as the 4‰ initiative for increasing soil C storage in agricultural areas (Le Foll
518 2015) and efforts to optimize long-term biogeochemical functioning of agricultural peatlands.

519

520

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527

528

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718 TABLE 1. Characteristics of the different study areas (ID) used for PLFA or CLPP. Land
 719 management of peatlands is given as the percentage of the management style to the total
 720 peatland. Estimated N and P application through fertilizers (organic and inorganic) and the
 721 total water area, peaty soil and clay soil percentages are also shown for each area.

722

	ID	Lon (°)	Lat (°)	Int. Agr. (%)	AES (%)	Nat. (%)	Org. N fertilizer (kg/ha/y)	Org. P fertilizer (kg/ha/y)	Inorg. N fertilizer (kg/ha/y)	Inorg. P fertilizer (kg/ha/y)	Water Area (%)	Peat (%)	Clay (%)
PLFA	<i>H</i>	4.75494	51.88792	50	24	26	167.2	62.7	36.9	11.7	13	95	5
	<i>I</i>	4.82294	51.86355	63	35	2	151.1	56.7	79.7	23.3	10	100	0
	<i>O</i>	4.89699	51.91930	90	9	1	169.3	66.5	86.2	26.3	10	93	7
CLPP	<i>Q</i>	4.53776	51.75151	5	9	86	160.9	63.9	0.0	0.0	7	61	39
	<i>R</i>	5.00921	52.25942	58	36	6	169.0	67.7	75.7	21.8	14	80	20
	<i>S</i>	5.03050	52.19264	44	0	56	134.7	68.7	19.8	6.5	12	83	17
	<i>T</i>	5.13003	52.27827	28	0	72	136.5	68.4	14.7	4.6	19	94	4
	<i>U</i>	4.77225	51.93984	65	9	26	167.5	62.8	36.0	11.6	13	94	6
	<i>Z</i>	4.78264	52.03339	3	8	90	92.0	48.6	0.0	0.0	16	100	0

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724 TABLE 2. Average and range [min;max] of local soil conditions of the different areas (*H*, *I*, *O*)
 725 used in soil community structure analyses (PLFA).

Variables	<i>H</i>	<i>I</i>	<i>O</i>
<i>pH</i>	4.51 [3.91 ; 5.4]	4.93 [3.87 ; 6.64]	4.34 [3.70 ; 5.10]
<i>C</i> (mg/g dry weight of soil)	208.62 [158.86 ; 273.75]	196.48 [92.9 ; 240.64]	177.96 [105.13 ; 237.67]
<i>N</i> (mg/g dry weight of soil)	14.95 [11.59 ; 18.74]	14.68 [6.8 ; 18.04]	12.89 [7.79 ; 16.57]
<i>P</i> (mg/g dry weight of soil)	1.42 [0.75 ; 2.06]	1.33 [0.84 ; 2.01]	2.69 [2.04 ; 4.22]
Moisture (%)	64.63 [40.23 ; 76.97]	61.88 [49.96 ; 74.72]	62.56 [37.78 ; 77.43]
Microbial biomass (nmol/g dry weight of soil)	20.01 [4.81 ; 64.93]	14.04 [4.93 ; 31.42]	11.77 [3.45 ; 38.35]
<i>FB_ratio</i> (-)	0.06 [0.03 ; 0.09]	0.06 [0.03 ; 0.09]	0.06 [0.03 ; 0.1]

726 TABLE 3. Average and range [min;max] of local soil conditions of the different sampling areas
 727 (Q,R,S,T,U,Z) used in soil community functioning analyses (CLPP).

Variables	Q	R	S	T	U	Z
<i>pH</i>	6.67 [4.74 ; 7.84]	5.44 [4.76 ; 6.4]	5.34 [4.23 ; 6.62]	5.30 [4.06 ; 6.24]	6.03 [5.46 ; 7.43]	5.55 [4.63 ; 6.03]
<i>C (mg/g dry weight of soil)</i>	121.17 [59.6 ; 273.9]	228.0 [152.2 ; 284.1]	255.4 [167.7 ; 298.3]	156.51 [47.9 ; 410.49]	220.6 [27.2 ; 315.9]	268.9 [159.0 ; 329.0]
<i>N (mg/g dry weight of soil)</i>	8.23 [4.45 ; 17.46]	16.6 [12.12 ; 20.53]	19.55 [13.1 ; 22.18]	10.05 [3.66 ; 22.67]	16.54 [1.64 ; 23.06]	21.31 [14.29 ; 25.48]
<i>P (mg/g dry weight of soil)</i>	1.00 [0.39 ; 1.77]	1.28 [0.94 ; 2.07]	1.59 [1.21 ; 2.07]	0.91 [0.42 ; 1.28]	1.5 [0.75 ; 2]	0.72 [0.49 ; 1.15]
<i>Moisture (%)</i>	50.21 [14.83 ; 69.29]	68.62 [56.12 ; 87.12]	68.44 [55.4 ; 76.8]	58.13 [34.82 ; 80.83]	65.37 [31.54 ; 78.54]	72.33 [49.52 ; 79.41]
<i>Substrate utilisation (h⁻¹)</i>	0.0171 [0.006 ; 0.179]	0.0103 [0.005 ; 0.023]	0.010 [0.003 ; 0.025]	0.0085 [0.003 ; 0.020]	0.0102 [0.006 ; 0.018]	0.0107 [0.0038 ; 0.027]
<i>AWCD</i>	0.73 [0.55 ; 0.89]	0.64 [0.42 ; 0.9]	0.73 [0.48 ; 0.89]	0.5 [0.2 ; 0.78]	0.65 [0.47 ; 0.88]	0.64 [0.24 ; 0.99]
<i>Substrate richness</i>	27 [22 ; 31]	24.38 [16 ; 31]	26.5 [23 ; 31]	24.79 [19 ; 30]	25.62 [21 ; 30]	25.75 [19 ; 30]
<i>Substrate diversity (Shannon)</i>	22.78 [17.59 ; 25.54]	20.62 [13.48 ; 25.21]	22.56 [19.2 ; 26.72]	19.77 [14.23 ; 25.53]	21.18 [17.26 ; 27.82]	20.91 [14.45 ; 26.77]
<i>Substrate evenness</i>	0.71 [0.55 ; 0.8]	0.64 [0.42 ; 0.79]	0.7 [0.6 ; 0.84]	0.62 [0.44 ; 0.8]	0.66 [0.54 ; 0.87]	0.65 [0.45 ; 0.84]

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739 TABLE 4. Importance of variables underlying soil microbial community structure (PLFA) at
 740 both scale levels (local and landscape).

Model	Variable	Explained variation*	
		Local	Landscape
<i>Soil characteristics (SOIL)</i>	Soil P content	-	10.3
	Soil type: Organic top soil on deep peat layer	-	7.8
	Soil N content	-	1.7
<i>Nutrient management (NUT)</i>	Organic P fertilization	5.7	1.0
	Management: Nature - Moist meadow-bird grassland	0.8	2.6
	Inorganic N fertilization	-	3.1
	Organic N fertilization	-	0.6
	Management: AES - Meadow-bird nest protection	-	0.2
<i>Spatial patterns (SPACE)</i>	ns	-	-
<i>Vegetation composition (VEG)</i>	Vegetation composition	-	4.6

Footnotes

- * Explained variation of each variable is given as R² (%) of the variable
- Variable was not selected in the forward selection of the specific model

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743 TABLE 5: Importance of variables underlying soil microbial functional capacity (CLPP) at
 744 both scale levels (local and landscape).

Model	Variable	Explained variation*	
		Local	Landscape
<i>Soil characteristics (SOIL)</i>	Soil pH	-	1.1
	Soil type: Sand	-	0.9
	Soil N:P	-	0.7
	Bank angle	-	0.6
	Soil C:P	-	0.6
	Soil type: Clay on peat	-	0.6
	Soil N content	-	0.6
	Soil C:N	-	0.3
<i>Nutrient management (NUT)</i>	ns	-	-
<i>Spatial patterns (SPACE)[†]</i>	MEM1	7.7	-
	MEM2	3.1	-
	MEM3	1.4	-
<i>Vegetation composition (VEG)</i>	Vegetation composition	8.7	2.2

Footnotes

- * Explained variation of each variable is given as R² (%) of the variable
- Variable was not selected in the forward selection of the specific model
- † Spatial patterns model is composed of Moran Eigenvector Map (MEM) variables based on geographical distance as per Dray et al., 2006. Variables of increasing order indicated decreasing scale of spatial patterning.

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747 FIG. 1. Overview of sample areas and sample sites. a) The Netherlands with the studied region
748 indicated in an orange rectangle. b) Map with the areas sampled in 2013 for PLFA analyses
749 (orange) and in 2014 for CLPP analyses (green). c) Detailed map of one of the study areas
750 indicating the location of the 24 sampling sites for this specific area. d) Schematic
751 representation of how samples were collected along the waterside of ditches.

752 FIG. 2. Estimated standardized coefficients for AIC selected generalized linear models of
753 total, fungal, bacterial, G⁺ bacterial and G⁻ bacterial biomass explained by environmental
754 drivers. Asterisks indicate significant coefficients (*** p < 0.001; ** p < 0.01; * p < 0.05.)
755 with a coefficient above 1 being a positive and below 1 a negative correlation with the
756 biomass component.

757

758 Fig. 3. PCA plots of soil microbial structure data (PLFA) for the three different groups of
759 drivers (Soil characteristics, Nutrient management and Vegetation) and shifts in total
760 microbial biomass, fungi, gram-positive bacteria, gram-negative bacteria and total bacteria.
761 Arrows are projected variables showing variables with the highest squared correlation
762 coefficients. Different colours indicate the different sampled areas (H, I, O).

763

764 Fig. 4. PCA plots of soil microbial functional capacity data (CLPP) for the three different
765 groups of drivers (Soil characteristics, Nutrient management and Vegetation), with
766 projections of the shifts in the utilization of specific substrate types. Arrows are projected
767 variables showing factors variables with the highest squared correlation coefficients. Different
768 colours indicate the different sampled areas (see Materials and Methods).

769

770 FIG. 5. Drivers of microbial community structure and functioning on local and landscape
771 scale. Venn diagrams showing the variation partitioning of different statistical dbRDA

772 models: a soil characteristics model (SOIL), a nutrient management model (NUT) and a
773 vegetation composition model (VEG) and a spatial model (SPACE). These models are used to
774 explain soil microbial structure (PLFA) at the landscape (a) and local (b) scale, and
775 functioning (CLPP) at the landscape (c) and local scale (d) by different drivers. Stars indicate
776 significance and numbers express the adjusted R^2 (%) of the model partitions.

777

778 FIG. 6. Schematic representation of the effects of reduced environmental quality on soil
779 microbial community structure and functioning. This conceptual figure illustrates how reduced
780 environmental quality of drivers relevant for functional capacity will directly lead to shifts of
781 soil microbial functioning away from its desired function. Reduced environmental quality
782 relevant for microbial structural composition will cause shifts in the soil microbial community
783 structure box. This can ultimately also result in a shift in soil microbial community function
784 through its constraint on microbial function. Within the context of the current study, the
785 environmental drivers of microbial functioning were found to be manifest at the local scale,
786 while the drivers shaping structure operated at the landscape scale.

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