Microbial landscape ecology: landscape structure better predicts soil protist diversity than local edaphic variables in alpine meadows

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April 11, 2022

Abstract

Context: Human-induced changes in landscape structure are among the main causes of biodiversity loss. However, despite their important contribution to biodiversity and ecosystem functioning, microbes - and particularly protists - remain spatially understudied. Furthermore, soil microbiota are most often related to the local soil properties, whereas the influence of the surrounding landscape is rarely assessed, calling for microbial landscape ecology studies.

Objectives: Here, we assessed for the first time the effect of changes in landscape composition and configuration on soil protist diversity in meadows in the Swiss western Alps.

Methods: We sampled 178 plots along an elevation gradient representing a broad range of climatic, edaphic, and land use conditions, and measured landscape structures in several neighbourhood windows of increasing size.

Results: Our results showed that landscape structures between 500 m and 2000 m around plot centres better predicted microbial diversity than local edaphic and climatic descriptors, with the percentage cover of open habitat and meadows and, to a lesser extent, edge density being the most important descriptors. The patch level descriptors explained the least amount of variation in protist diversity, what is in contradiction with macro-organims where patch size and shape strongly impact the population dynamics. This study also highlights different responses of protist functional groups to changes in landscape structure.

Conclusions: Our results importantly show the significance of landscape structure for microbial diversity and the potentially negative consequences of land use change on soil microbial communities and their associated functions.

Key-words: protist diversity, landscape ecology, functional diversity, grassland, Swiss western Alps, soils

1 Introduction

Land-use change is one of the main causes of terrestrial biodiversity loss (Brondizio et al., 2019; Cardinale et al., 2012; Mace et al., 2018; Sala et al., 2000). Over the past centuries, about 75% of the global land area has been impacted by human activities (Ellis and Ramankutty, 2008). These changes have modified landscape structure through destruction, degradation, and/or fragmentation of natural habitats with negative consequences on local biodiversity and the associated ecosystem functions and services (Brondizio et al., 2019). Anthropogenic modifications of landscape structure are likely to continue in the future to meet the increasing need of a growing human population (Tilman et al., 2011; Mace et al., 2018). In this context, understanding the impact of changes in landscape structure on ecosystems is required to maintain landscape biodiversity and services.

Microbes represent the dominant part of biodiversity on Earth (Locey and Lennon, 2016). They are also central actors to key ecosystem functions and services including carbon sequestration (Shakoor et al., 2020), litter decomposition (Jackson et al., 2017), nutrient turnover (Wagg et al., 2014), food production (Nosheen et al., 2021), water quality supply (Sagova-Mareckova et al., 2021) or regulation of biological populations (Kibblewhite et al., 2008). However, despite their important contribution to biodiversity and ecosystem functions, microbes are rarely considered in spatial studies assessing the impact of environmental conditions on biodiversity (as e.g. Malard et al., 2021; Mod et al., 2020, 2021; Seppey et al., 2020), and landscape structure is considered even less frequently (Mennicken et al., 2020; Mony et al., 2020). As a result, the importance of landscape structure for local microbial diversity and functionality remains unclear. This is a major knowledge gap in microbial ecology as well as in our understanding of the consequences of land use changes.

Studies analysing the drivers of microbial diversity have primarily focused on local processes assuming that microbial community composition results from the effect of the local abiotic environment and from biotic interactions (Louca et al., 2018; Mod et al., 2020; Yashiro et al., 2018). These studies have shown the importance of environmental conditions such as temperature (Fierer et al., 2006; Mod et al., 2020), pH (Chu et al., 2010; Yashiro et al., 2016), and soil moisture (Sofi et al., 2016). Other studies have focused on biogeographical processes revealing patterns of microbial diversity (Martiny et al., 2006), such as continental divide (Glaeser and Overmann, 2004; Louca, 2021), latitudinal gradient (Schwalbach and Fuhrman, 2005), or distance-decay relationships (Hanson et al., 2019; Schwob et al., 2021). However, these studies have mostly ignored the impact of regional-scale processes on local community composition (Leibold et al., 2004; D'Amen et al., 2017). Because landscape structure modulates the strength and type of impact of regional processes on local communities (Fournier et al., 2017), modern landscape ecology approaches (e.g. **?**) have the potential to shed new light on the processes driving microbial community assembly, and particularly on the importance of dispersal from a regional pool of potential colonists.

Despite their potential, landscape ecology approaches applied to microbial communities remain scarce (Mony et al., 2020), mostly due to the difficulty to observe these organisms. Progress in DNA-based characterization of community composition now makes it easier to conduct landscape-scale surveys of microbial diversity (Kibblewhite et al., 2008). However, the few landscape ecology studies focusing on microbes are taxonomically and functionally biassed towards parasitic organisms and pathogens, mostly fungi or bacteria (Cruz-Paredes et al., 2021; Mony et al., 2020). This line of research demonstrated the importance of landscape structure such as roads for the dispersal and distribution of pathogens (e.g. Bousset et al., 2018; Holdenrieder et al., 2004; Laine and Hanski, 2006). Yet, to our knowledge, the effect of landscape structure on microbial communities at large taxonomic scale (here, protists) has never been assessed. Because of the key functional importance of soil protists (Geisen et al., 2016; Seppey et al., 2017; Xiong et al., 2018), this gap limits our understanding of the consequences of land use change on ecosystem structure and function.

The present study evaluates the effect of landscape structure on local soil protist diversity across 178 non-forest open vegetation plots (mostly meadows) in the Swiss western Alps. We assess the effect of landscape descriptors related to landscape composition (i.e. the relative proportion of land cover types in the landscape, regardless of spatial distribution) and configuration (i.e. the spatial arrangement of land cover types in the landscape) on local microbial diversity and compare it to edaphic and topo-climatic descriptors. We hypothesise that (1) landscape descriptors will provide complementary information to local abiotic conditions, thereby improving our capacity to predict microbial diversity. We further hypothesise that (2), because of their strong habitat specificity and high dispersal capacity, soil protist diversity will be more sensitive to landscape composition than to configuration as observed in larger-sized organisms (Betts et al., 2019; Martin et al., 2019). Because many microbes are good dispersers (Lara and Belbahri, 2011; Louca, 2021) we expect (3) dispersal from neighbouring habitats to significantly influence local microbial diversity. As a result, landscape descriptors related to edge effects should be important descriptors of soil protist diversity. We also assess the relative influence of various landscape components across protist functional and taxonomic groups. Altogether, we expect these results to reveal the significance of land use change on

soil microbes' ecology and, consequently, on the related ecosystem functions and services.

2 Material and Methods

2.1 Study site and sampling design

We focus primarily on alpine meadows in the Swiss Western Alps (Fig. S1). As in most Western European mountain systems, meadows are threatened by both urbanisation and agricultural abandonment allowing forests to take over. As a result, the area occupied by meadows is decreasing and remains are increasingly fragmented (Dengler et al., 2014). These changes in landscape structure impact plants (Dengler et al., 2014) and, expectedly, protist diversity, and are likely to increase in the future (Pellissier et al., 2013). For these reasons, alpine meadows in the study area constitute a useful model ecosystem to study the impact of landscape structure on soil microbial communities.

A total of 178 non-forest open vegetation plots (each of 2 m by 2 m) were sampled in the study area from July 4th to September 1st 2013 according to a sampling design stratified by altitude. A total of 208 samples were recovered as some plots were sampled multiple times. For each sample, five 100 g soil cores were taken (from the four corners and centre of the plot, respectively, i.e. one 'sample'). The soil cores were then mixed and kept below 4 °C until further processing. For more details, see Buri et al. (2020) and Yashiro et al. (2016).

2.2 Edaphic descriptors

For each sample, 33 edaphic descriptors were measured (Table S1). Relative humidity (rh) and soil organic carbon (LOI) have been measured gravimetrically. Percentages of sand, silt, and shale were measured by laser granulometry. Electroconductivity (EC) and pH were measured in a soil and Milli-Q water slurry. Total phosphorus was measured by colorimetry after decarbonization. C/N ratio was calculated from total carbon and nitrogen content measured from spectroscopy (Carlo Erba CNS2500 CHN elemental analyzer). Total organic carbon (TOC) and mineral carbon (MINC) were measured by pyrolysis. The mineralogy was determined by X-Ray powder diffraction and fluorescence. A description of these analyses can be found in Buri et al. (2020) and Yashiro et al. (2016).

2.3 Topo-climatic descriptors

Topo-climatic descriptors were derived from data of the Swiss Meteorological Network (resolution 25 m; average trends for the period 1961-1990; `https://www.meteoswiss.admin.ch`) and Swisstopo (resolution 25 m `https://www.swisstopo.admin.ch/`). The descriptors were elevation (mnt25), slope steepness (slope), slope orientation (asp25) and the sinus of the orientation (aspvar), topographic position (topo), topographic wetness index (twi25), site water balance (swb), shortwave radiation per month (sumradyy), monthly mean precipitation sum (precyy), number of precipitation days per growing season (pday), monthly moisture index (mmind68), monthly average temperature (taveyy), annual degree-day above 0 °C (ddeg0), and number of frost days during the growing season (sfroy)(Buri et al., 2020; Mod et al., 2020, 2021; Pradervand et al., 2014; Yashiro et al., 2016, 2018). In addition to these descriptors, bioclimatic descriptors were added from the Chelsa database (Karger et al., 2017) (Table S1).

For both edaphic and topo-climatic descriptors, the few missing values (1 rh, 4 spectroscopy, 3 mineralogy, 6 C/N when nitrogen content was below the detection limit) were inferred by nearest neighbour averaging (function `preProcess`, method 'knnImpute', package `caret` v. 6.0-86 Kuhn, 2020). The dimensionality of the edaphic and topo-climatic datasets was reduced using a principal component analysis. In order to capture 80% of the total variance in each dataset, we retained the first ten and the first four PCA axes for edaphic and topo-climatic descriptors, respectively (Fig. S2).

2.4 Landscape descriptors

The landscape descriptors have been calculated based on the 10 m resolution land cover raster derived from Sentinel-2 imagery (Malinowski et al., 2020) (`http://s2glc.cbk.waw.pl/`). This raster includes 13 land cover categories such as coniferous forest (representing 27.2% of the study area), herbaceous vegetation (25.6%), moors and heathlands (16.2%), and broadleaf tree forest (11.0%). A total of 13 descriptors commonly used in landscape ecology studies and representing different aspects of landscape configuration and composition were computed (see Table 1 for details). To explore the spatial scale at which landscape structure impacts soil protists diversity, each descriptor was calculated in various circular areas of different radius around the sampling coordinate (buffer size = 100, 200, 500, 1000, and 2000 m; Fig. S3).

Table 1: list of the selected landscape descriptors

Abbreviation	Descriptor	Level	Explanation (units)
area	sampling patch area	patch	(hectares)
area_mn	average size of meadow patches	class	(hectares)
%_meadow	% of meadows	class	% of herbaceous vegetation within the buffer
%_anthro	% of built-up habitat	class	% of artificial surfaces i.e. constructed and cultivated areas within the buffer
%_forest	% of forests	class	% of broadleaf and coniferous tree cover within the buffer
%_open	% of open habitat	class	% of herbaceous vegetation, moors, heatland, sclerophyllous vegetation, marshes, and peat bogs within the buffer
frac	fractality	patch	complexity of the sampling patch
cai	core area index	patch	area of the sampling patch minus the pixel touching an other patch (percehtage)
ed	edge density	class	sum of all meadow edges divided by the area of the raster (metres per hectare)
iji	interspersion and juxtaposition index	index	intermixing of meadow within other habitat classes (percent)
clumpy	clumpiness	class	calculate the disaggregation of the meadows
division	division	class	probability that two meadow points are not in the same patch
shdi	Shannon diversity index of habitat	landscape	descriptor increasing according to the number of landscape classes and evenness of these classes

2.5 Protist community

2.5.1 DNA extraction, PCR, sequencing

DNA was extracted from 0.25 g of soil samples in triplicates with the MoBio PowerSoil DNA extraction kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The V4 region of the 18S rRNA gene was then amplified using the general eukaryotic primers TAReuk454FWD1 and TAReukREV3 (Stoeck et al., 2010). PCR amplification details can be found in Seppey et al. (2020). Paired-end reads (2 * 300 bp) were then sequenced from an Illumina MiSeq sequencer at the University of Geneva (Molecular Systematics & Environmental Genomics Laboratory) as in Seppey et al. (2020). Sequences are available on the European Nucleotide Archive under the project PRJEB30010 (ERP112373).

2.5.2 Bioinformatic pipeline

The 208 samples provided 128'581'740 raw reads that were analysed with a pipeline based on `vsearch` v. 2.15.2 (Rognes et al., 2016). The reads were first merged with `Flash` v. 1.2.11 (Magoc and Salzberg, 2011) before removing the sequences shorter than 250 nucleotides. Reads with a quotient between expected error and sequence length below 0.01 as well as chimera were discarded. The 23'486'785 reads left (18%) were then clustered into 1'215'009 OTUs with the `Swarm` algorithm using the fastidious option (v. 3.1.0, Mahé et al., 2014).

2.5.3 The taxonomic assignment of the OTUs was performed in two steps.

The first part was aiming to identify and remove the OTUs not belonging to protists (i.e. Metazoa, Embryophyta and Fungi). This was done by using the best alignment from a pairwise local alignment (`blastn` v. 2.9.0, Camacho et al., 2009) of the OTUs dominant sequences on the pooled PR^2 4.12.0 (Guillou et al., 2013) and Silva v. 138 (non-redundant at 99 % Pruesse et al., 2007) databases. The second part consisted of the final assignment of the remaining OTUs by aligning their dominant sequences against the PR^2 database v. 4.12.0 using the best alignment of a global pairwise alignment (`ggsearch36` v. 36.3.8, Pearson, 2000). Prior to the alignment, the PR^2 database was trimmed according to the primers used for the sequencing after a multiple sequence alignment performed with `ClustalW` v. 2.1 (Larkin et al., 2007).

From the 178 plots, 4 were sampled twice and 13 three times during the sampling period. For each of these plots, OTUs abundance was estimated as the median of the number of sequences retrieved from the different samples. In addition, 16 plots with a low number of sequences were removed by using the breaking point of a piecewise linear model on the sorted log of the number of sequences per sample (threshold = 6047 sequences) (Fig. S4). In total, 162 plots were kept for further analyses. We then discarded the OTUs with near-zero variance (ratio between the two most frequent number of sequences per OTU below 95/5, and unique number of sequence within a OTU below 10% of the number of samples; function `nearZeroVariance`, package `caret` v. 6.0-86 Kuhn, 2020). OTUs were also assigned to one of the three main eukaryotic functional groups: consumers, parasites, and phototrophs according to their taxonomic position based on Adl et al. (2019). The final dataset included 162 plots and 3992 protist OTUs.

2.6 Statistical analyses

The Shannon diversity index was calculated for the overall community as well as for each dominant soil group: Apicomplexans and early-branching Alveolata (Colpodellida, Eugregarinorida, Neogregarinorida), Ciliates (Colpodea, Litostomatea, Oligohymenophorea, Spirotrichea) Amoebozoa (Variosea, Lobosea), Apusomonadida, Archaeplastida (Chlorophyceae, Trebouxiophyceae), Rhizaria (Granofilosea, Imbricatea, Cercomonadida, Glissomonadida, Thecofilosea) and Stramenopiles (Chrysophyceae, Oomycota, Labyrinthulomycetes).

A random forest algorithm was used to model the response of soil protist diversity to landscape and local edaphic and topo-climatic descriptors. Several runs of random forest were conducted within a 10-fold cross-validation stratified according to the response variable, repeated three times. For each descriptor, the importance of the descriptors (function `varImp`, package `caret` v. 6.0-86 (Kuhn, 2020)), as well as partial dependence plots (Goldstein et al., 2015, function `partial`, package `pdp` v. 0.7.0 Greenwell, 2017), were extracted from the model. Descriptor importance was estimated as the residual sum of squares. To allow comparisons among models, the importance of each descriptor was weighted by the R^2 of the model. This procedure was repeated for each diversity descriptor (overall, functional groups, and taxonomic groups) and spatial buffers (N=24 Shannon diversity indices * 5 spatial buffers=120 models).

3 Results

3.1 Protist community composition

Soil protist diversity was dominated by Alveolata and Rhizaria, which represented 37% and 33% of the sequences, respectively. The sum of the selected 20 dominant taxonomic groups represented 78% of all reads and 76% of the OTUs. Consumers represented 66.5%, parasites 26.3%, phototrophs 6.3%, of the considered reads, respectively. Fig. S5 provides further information about protist community composition.

3.2 Influence of landscape structure on soil protist diversity

The amount of variation in protist diversity explained by landscape descriptors was higher than that explained by edaphic and topo-climatic descriptors when using larger neighbourhood windows (500 m to 2000 m around plot centre) and lower when using smaller buffers (100 m and 200 m) (Fig. 1A). The percentage of open habitats around a plot explained the highest amount of variation among all landscape descriptors and was overall the third best descriptor (Fig. 1B). Half of the ten most important predictors were landscape descriptors, namely %_open, ed, %_meadow, division, %_forest, and area_mn. Patch level descriptors (patch area, patch core area, and patch fractality) explained the lowest amount of variation among all considered descriptors. Focusing on the three landscape descriptors that explained the largest part of soil protist diversity, total soil protist diversity was positively correlated with %_open and followed a unimodal relation with ed and %_meadow (Fig. 1C). Other important descriptors included area_mn (500 m), division (500 m; an indicator of the degree of fragmentation of the landscape), and the percentage of forest around the plot (2000 m) (Fig. 1B). In addition to landscape descriptors, soil protist diversity was mostly correlated with precipitation regime (tocl_PC3), mineralogy (edap_PC9), carbon and calcium content (edap_PC1), temperature (tocl_PC1), and granulometry (edap_PC6) (Fig. 1B, Table S2, S3).

3.3 Functional and taxonomic groups

Landscape descriptors were also important predictors of the Shannon diversity within each functional group (Fig. 2). Consumers and parasites responded similarly to landscape structure, showing similar responses to the three landscape descriptors explaining the most variation of overall protist diversity (Fig. 1D-E). They presented a positive relationship with the percentage of forest (500 m) and a peak of diversity at intermediate values of percentage of meadows (1000 m) and edge density (500 m). Furthermore, the five landscape descriptors explaining the most variation in the models were the same in both functional groups (Fig. 2). Conversely, phototrophic protists showed the lowest diversity values at intermediate percentages of open habitats (500 m) and decreasing diversity with increasing percentages of meadows (1000 m) and edge density (500 m) (Fig. 1F). The most important landscape descriptors for this group were different than for protist diversity with the Shannon diversity of land cover types (2000 m) and the area of forests (2000 m) explaining the most variation in phototroph diversity (Figs. 2, S9). All functional groups also shared similar patterns to protist diversity regarding buffer size and patch level descriptors. For the three functional groups, landscape descriptors calculated within the smallest buffers (100 m and 200 m) generally explained a lower amount of variation than those calculated in larger buffer, and patch level descriptors (patch area, patch core area, and patch fractality) explained the lowest amount of variation among all considered descriptors. We also observed significant variability in the overall response of individual taxonomic groups to landscape structure with two amoebozoans (Lobosea and Variosea) and a ciliate (Colpodea) taxa being the most strongly impacted by landscape descriptors (Fig. S10).

4 Discussion

We showed the importance of landscape structure in explaining microbial diversity, here illustrated with soil protists. This adds to the body of evidence showing the importance of spatial environmental factors such as topography, and climate (Seppey et al., 2020) in structuring protist communities and provides new insights on the potential effects of land use change. Models based on landscape descriptors calculated in the larger neighbourhood windows (buffers of 500 m to 2000 m) explained a larger part of the variation in protist diversity than models based on edaphic and topo-climatic descriptors alone. This confirmed that landscape descriptors provide valuable complementary information to local abiotic conditions (hypothesis 1). The opposite trend was observed for smaller buffers, highlighting the importance of scale in microbial landscape ecology (Cadotte, 2006; Chalmandrier et al., 2019; Lembrechts et al., 2020) as reported for macro-organisms (Bradter et al., 2013; McGill, 2010). In agreement with hypothesis 2, soil protist diversity was more sensitive to landscape composition (i.e. the relative proportion of land cover types in the landscape, regardless of spatial distribution) than to configuration (i.e. the spatial arrangement of land cover types in the landscape). Nevertheless, edge metrics were important descriptors of protist diversity confirming that dispersal from neighbouring habitats influences local microbial diversity (hypothesis 3; Berga et al., 2015; Livingston et al., 2013; Thrall et al., 2003). There was also an important variability among functional and taxonomic groups suggesting that landscape-scale processes are, at least to some extent, functional group / taxon-specific.

4.1 Landscape composition and configuration influence local microbial diversity

Overall our results highlight the key role of landscape structure for soil protist diversity in alpine meadows. Landscape composition descriptors (e.g. percentage of open habitats or meadows around each plot) had the most important effect on soil protist diversity. On the contrary, landscape configuration descriptors (e.g. division, interspersion and juxtaposition index), although important, contributed less. This agrees with the 'habitat amount hypothesis'

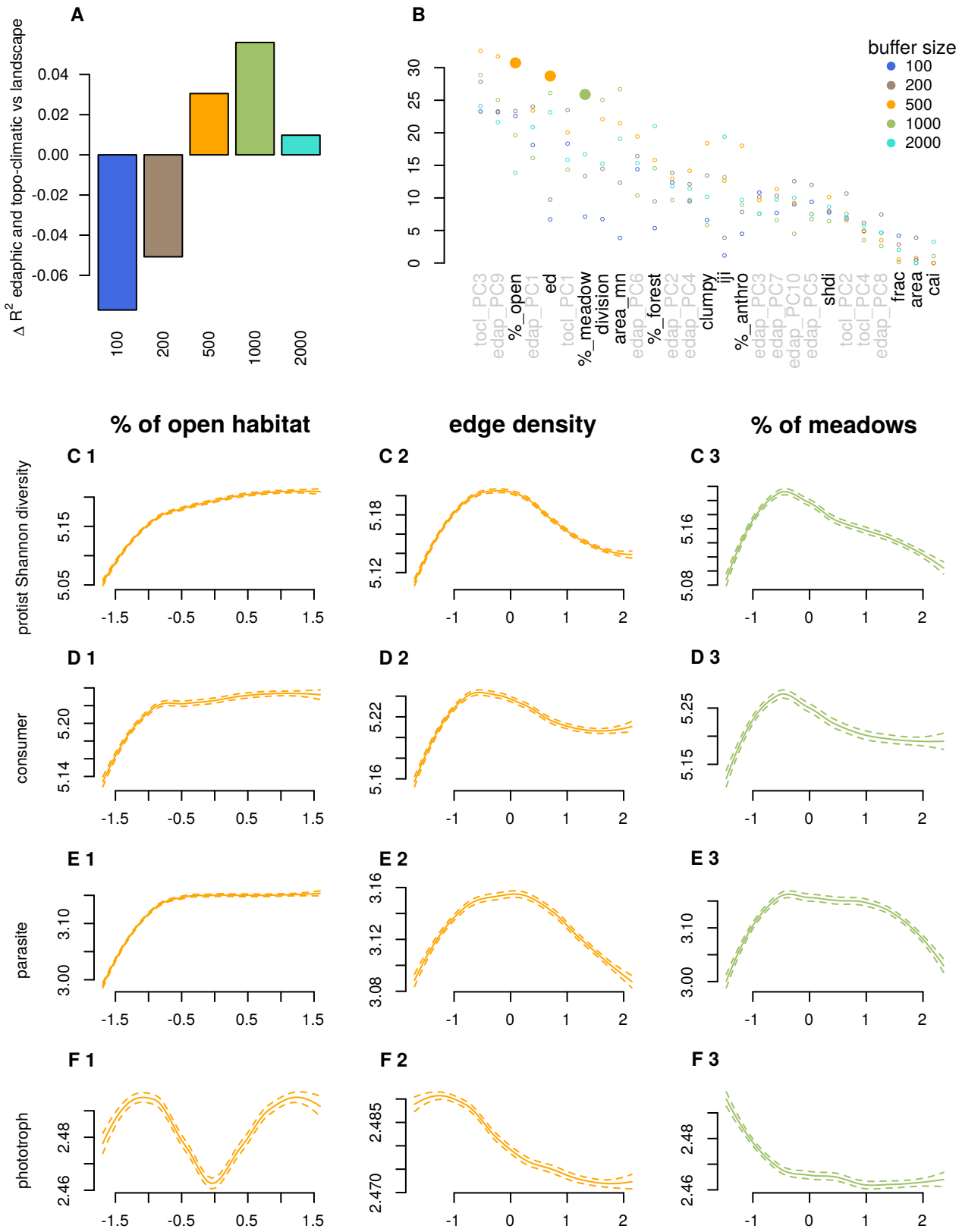

Figure 1: Effect of landscape structure on soil protist diversity. (A) Difference between the variation in total protist diversity (R^2) explained by landscape descriptors versus edaphic and topoclimatic descriptors in random forest models. (B) Variability in the importance of landscape and local descriptors for the prediction of overall soil protist diversity in random forest models. The importance has been weighted by the R^2 of the model to ease the comparison among models. Landscape descriptors are shown in black and topoclimatic and edaphic descriptors in grey. The three landscape descriptors with the highest importance are highlighted. (C-F) Partial dependence plots showing the trends of soil protist diversity along the three most important landscape descriptors. For each sub-figure, the y-axis reflects the effect that the target landscape descriptor (centred and scaled on the x-axis) has on the predicted values of the response variable while keeping all other descriptors constant. (C) Total soil protist diversity. (D) Consumer diversity. (E) Parasite diversity. (F) Phototrophic diversity. Colours indicate the different buffer sizes.

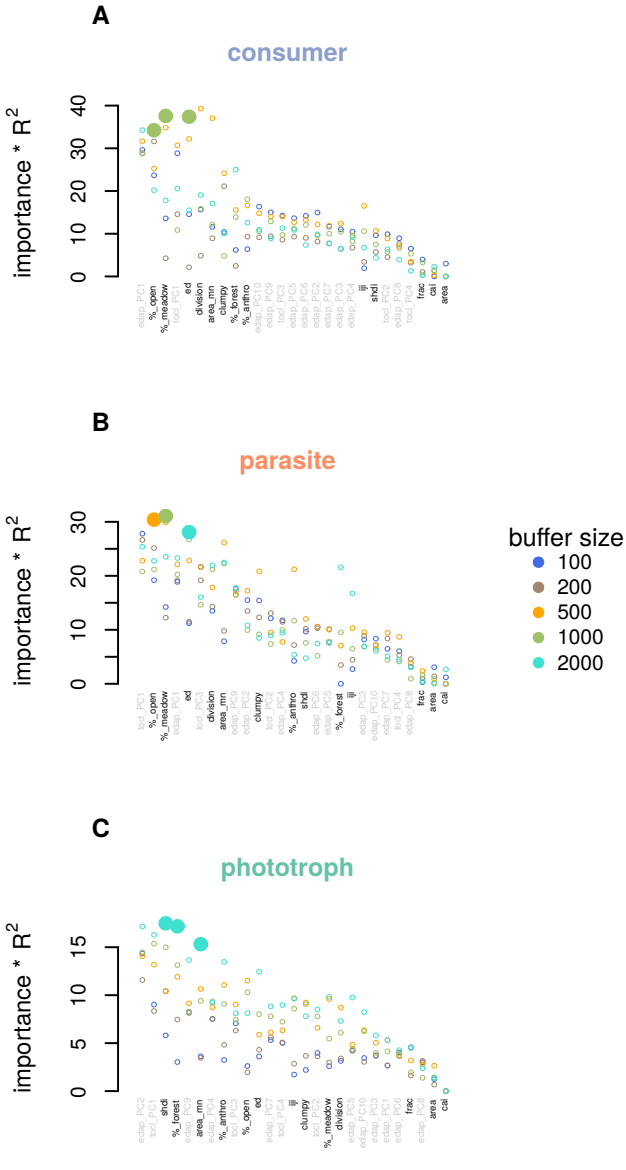

Figure 2: Importance of landscape, edaphic and topo-climatic descriptors on protist functional diversity. The importance has been weighted by the R^2 of each model to ease the comparison between the models within a functional group. For each descriptor, the colour of the dot represents the different buffer sizes. Landscape descriptors are shown in black while the edaphic ones are shown in grey.

that suggests that the proportion of habitat type in the landscape is more important than its spatial distribution (Fahrig et al., 2015; Laine and Hanski, 2006). These conclusions are similar to those of previous works on the distribution of fungi in old forests (Mennicken et al., 2020), suggesting that similar mechanisms impact protists and fungi following land use change.

In our study, a high proportion of open habitats around the plot had a positive effect on local diversity whereas a high proportion of built-up habitats had a negative one. Increasing proportion of forest habitats around a meadow contributed positively to local diversity up to a threshold where this positive effect saturated (i.e. further increase of forest area does not add to local diversity). This can be explained by different and richer communities in meadows as compared to forests (Seppey et al., 2017) or, expectedly, to built-up habitats. These results suggest that local protist composition and functional diversity depend on the taxonomic composition of the regional pool, which is itself determined by habitat composition in the landscape. Changes in landscape composition around a plot are thus likely to modify the functionality and resilience of local soil microbial communities (Saleem et al., 2019). As soil protists are key players in soil foodwebs (Xiong et al., 2018), these changes can potentially have a cascading impact on ecosystems functioning and services such as fertility. It is thus likely that the ongoing agricultural abandonment and urbanisation in the Alps (Vannier et al., 2016) will lead to a decrease in protist diversity in meadow soils with important consequences for the functioning of these ecosystems.

Despite the higher influence of landscape composition on protist diversity, our results also point towards strong edge effects. Edges are hotspots of biodiversity because of the presence of species associated with different habitats alongside ecotonal species (Descombes et al., 2017). They are also an important source of dispersal to neighbouring habitats (Molnar et al., 2001). In microbial ecology, only a few studies have investigated community composition in transition zones between habitats. For example, Boeraeve et al. (2019) showed that both distance to the forest edge and edge orientation to the south influence mycorrhizal communities of trees (Alnus glutinosa). In our study, soil protist diversity was maximal at intermediate edge density. A low edge density means a lower likelihood that colonists from adjacent habitats settle down in the meadow. Ecotonal specialists might also be lacking when edge density is too low. The lower local protist diversity at high edge density can tentatively be explained by two mechanisms. On the one hand, high edge density might favour a few generalist species over specialist taxa. Generalists can survive in various habitats and are more likely to benefit from highly mixed landscape habitat mosaics. On the other hand, edge density was positively correlated with the area of urban habitat (Spearman correlation $p < 0.001$, $\rho = 0.44$). As these habitats have a negative contribution to local diversity, soil protist diversity is lower than when only habitats with positive contributions to diversity are present. In this case, edge density would reflect a compositional effect rather than a true edge effect. Overall, maintaining sufficient landscape diversity in the Alps while avoiding excessive fragmentation is thus likely to have a positive impact on meadow soil protist diversity and functionality as observed in macroorganism groups such as orthoptera (Essl and

Dirnboeck, 2012), plants and pollinators (Jones et al., 2019).

4.2 The importance of scale and dispersal for the response of microbes to landscape structure

Our results show that landscape descriptors calculated in the larger neighbourhood windows (buffers of 500 m to 2000 m) have a stronger influence on local community composition than landscape descriptors calculated in smaller windows (100 to 200 m). Such results suggest the importance of dispersal in protist community dynamics as observed in experimental studies (Berga et al., 2015; Livingston et al., 2013; Thrall et al., 2003). Our results, in agreement with the study of Barnett et al. (2020) on bacteria, confirm that dispersal from neighbouring habitats is one of the main drivers of microbial community functional composition and diversity. Our results also highlight that the effect of dispersal depends on the taxonomic composition of the pool of potential colonists (caused by differences in landscape composition) which can explain the inconsistencies in the effect of dispersal pointed at by the review of Langenheder and Lindstrom (2019). These results are corroborated by the observations of Jauss et al. (2021), who found no dispersal limitations for protists over short distances. It is likely that strong landscape-scale dispersal prevents a local community from functioning in isolation. Microbial community dynamics should thus be considered at the landscape-scale using a metacommunity perspective (Beisner et al., 2006; Cadotte, 2006; Leibold et al., 2004) which has important consequences for land use management in the Alps and in general.

In addition, patch-scale descriptors had a consistently low influence on soil protist diversity. Here again, our results resemble previous observations showing that fungal richness is positively influenced by the area of old forests in the landscape and not by the focal patch size (Mennicken et al., 2020). While these convergent lines of evidence suggest that these conclusions are valid for all soil microbes, they are in strong contradiction with studies focusing on plants and animals, where patch size and shape have strong effects on local population dynamics (Prevedello and Vieira, 2010; Reed, 2004). This difference between macro- and microorganisms can be explained by differences in population size. Indeed, for the same area, the population size of microbes is larger by several orders of magnitude than that of macroorganisms. As such, population size fluctuations and genetic drift (and other population processes) are less likely to be problematic for microbes at the scale of a meadow.

4.3 Similarities and differences in the responses of protist functional groups

We observed important similarities in the effect of landscape structure among functional groups. For instance, all functional groups responded more strongly to habitat composition than configuration. In addition, all functional groups showed a strong response to landscape descriptors calculated in the larger buffers (500 m to 2000 m) and a weak response to patch level descriptors. However, our results also show that the response of phototrophs to changes in landscape structure differed from that of consumers and parasites. For instance, phototroph diversity tends to decrease with increasing proportion of open habitats and meadows in the landscape. This contrasts with the positive (open habitats) or unimodal (meadows) responses observed in consumers and parasites. These effects are probably explained by the existence of strikingly different communities between forests, meadows and anthropic habitats (Seppey et al., 2017). Phototrophic protists have differential abilities for modulating light energy input through accessory pigments (Perrine et al., 2012), which suggests that individual species have different light optima. As a result, species specific to closed ecosystems (forest and built-up habitats) might be absent locally if those habitats are lacking in the landscape. In agreement with this, phototroph diversity was maximal at intermediate proportions of built-up habitats, which contrasts with the negative response of the two other functional groups to the same variable (Figs. S7, S8, S9). Factors associated with anthropic habitats such as pollution are known to act negatively on consumer diversity as shown in ciliates, and simplify foodwebs (Lara et al., 2007).

Altogether, protist diversity in the Alps is favoured by a diversified landscape, containing equilibrated amounts of meadows and forests, but also to some extent built-up habitats. This stands in contrast with current tendencies towards increasing urbanisation and meadow recolonisation by forests as a result of agricultural abandonment (Spiegelberger et al., 2006). Diversified protist communities are key in many ecosystem services provided by soil, such as carbon storage and plant production (Geisen et al., 2019). The disappearance of traditional lifestyles in the Alps (Spiegelberger et al., 2006) can be expected to modify the structure and functioning of the soil microbial foodweb. Such modifications can potentially lead to more negative impacts on Alpine meadows than previously assessed based on macroorganisms alone (Spiegelberger et al., 2006).

4.4 Future developments of microbial landscape ecology

While the present study and several other recent works (Mennicken et al., 2020; Mony et al., 2021) provide a strong basis to further develop the field of microbial landscape ecology, much remains to be done. One of the main questions arising from our work is the real dispersal capacity of protists and microbes in general (Frey et al., 2013). The range of influence of a habitat (distance-decay in microbes at the landscape scale) and the influence of barriers to dispersal among the different microbial taxa (Paz et al., 2021; Wilkinson and Mitchell, 2010) needs to be investigated in the future. Likewise, questions arise about the generalisability of our results. Would we observe a similar relationship linking landscape and protist diversity in other habitats or regions (e.g. tropical areas)? Would other microbial groups show similar patterns (e.g. bacteria, fungi, archaea)? This last question is worth investigating, as, for instance, Mennicken et al. (2020) showed that bacteria and fungi responded differently to landscape composition. Finally, while our results highlight the importance of landscape structure for local protist community diversity and composition, the functional consequences of those changes and their magnitude remain unclear. Similarly, the extent to which these changes influence other taxa, including other microorganisms and macroorganisms, needs to be investigated.

Conclusion

Our results highlight the importance of landscape structure, especially changes in the composition of neighbouring habitats, for local soil microbial diversity. Landscape structure in the larger neighbourhood windows (buffers of 500 m to 2000 m) was even a better predictor of protist diversity than commonly used edaphic descriptors. We showed that, like macroorganisms, soil protists are strongly impacted by changes in landscape structure, which is likely to have important consequences on ecosystem structure and functioning. Using landscape ecology approaches in microbial ecology studies can improve our understanding of the drivers of and capacity to predict spatial patterns of microbial diversity. Microbial landscape ecology is a promising avenue to improve our understanding of the impact of land use changes on biodiversity and ecosystem functions.

Acknowledgements

BF and CVWS acknowledge the WISNA program from the German Federal Ministry of Education and Research. BF acknowledges the project "FunShift" (project FO 1420/1-1) awarded by the German Research Foundation, DFG. EL acknowledges the program "Atracción de Talento Investigador" from the Community of Madrid (project 2017-T1/AMB5210) and the project Myxotropic VI PGC2018-094660-BI00 awarded by the Spanish Government.

Authorship

The study was conceived by B.F. and C.V.W.S. and designed with input from all authors. Sampling design, fieldwork, and laboratory analyses were carried out by A.G., E.Y., and D.S.. C.V.W.S. carried out the bioinformatic and statistical analyses. B.F. and C.V.W.S. led the writing of the manuscript, all authors contributed to drafts of the manuscript and gave final approval for publication.

Data Accessibility Statement

All DNA sequences are available on the European Nucleotide Archive project number: PRJEB30010 (ERP112373). Edaphic variables are available from Dryad repository: `https://doi.org/10.5061/dryad.fttdz08p7`. Details about the topoclimatic data can be found at `https://www.unil.ch/files/live/sites/ecospat/files/shared/PDF_site/chclim25.pdf`

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Supplementary material

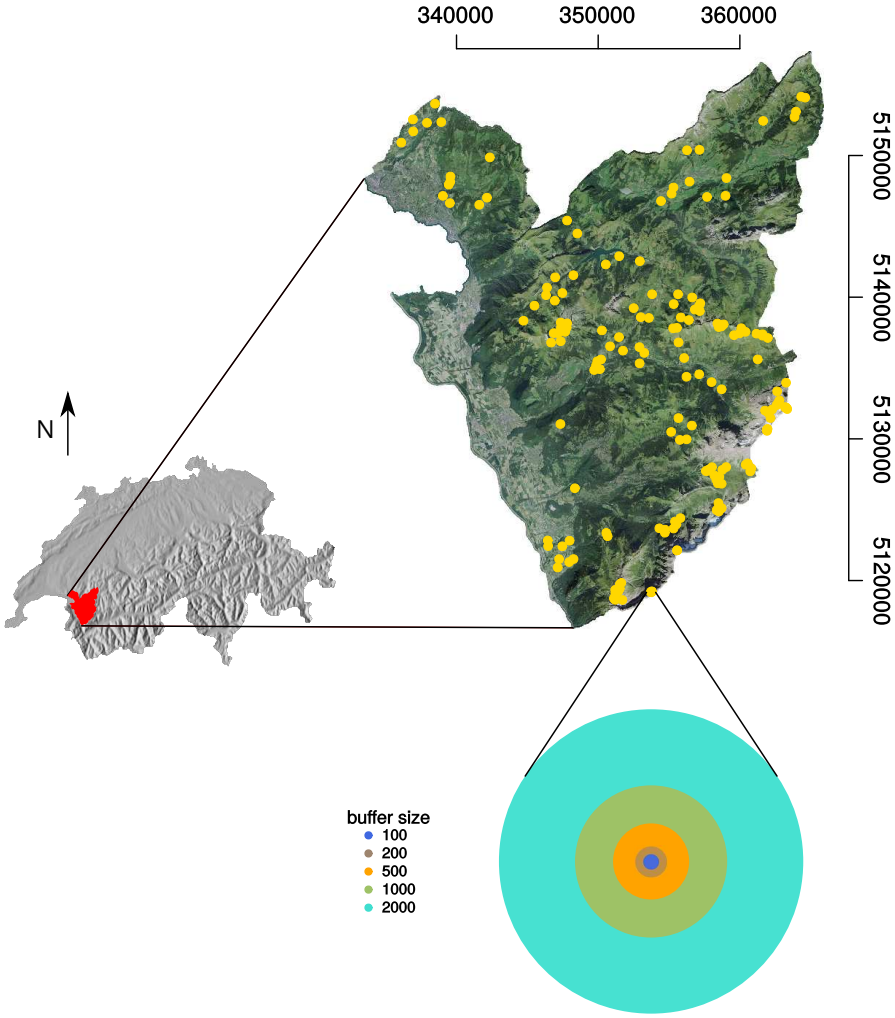

Figure S1: Map of the study area with 162 sampling locations (orange dots) in the Swiss Western Alps (coordinates are given as x and y Swiss coordinates). The lower panel shows the selected neighbourhood window around each plot (100, 200, 500, 1000, 2000 m).

Table S1: List of edaphic and topo-climatic variables and their abbreviations.

Edaphic variables		
Abbreviation	Variable	Unit
Soil_temp	temperature	°C
rh	relative humidity	%
LOI	soil organic carbon (loss on ignition)	%
EC	electroconductivity	µ S/cm
pH	pH	
P	total phosphorus	mg/g
C_N	C/N ratio	
Granulometry		
sand	percentage of sand	%
silt	percentage of silt	%
shale	percentage of shale	%
Pyrolysis		
TOC	total organic carbon	%
MINC	total mineral carbon	%
X-Ray mineralogy		
Phyllosilicates	Phyllosilicates	%
Quartz	Quartz	%
Feldspath_K	alkali feldspar	%
Plagioclase_Na	albite	%
Calcite	Calcite	%
Dolomite	Dolomite	%
Goethite	Goethite	%
Ankerite	Ankerite	%
Indoses	other	%
Diffraction mineralogy		
SiO2	silion dioxide	%
TiO2	titanium dioxide	%
Al2O3	aluminium oxide	%
Fe2O3	ferric oxide	%
MnO	manganese oxide	%
MgO	magnesium oxide	%
CaO	calcium oxide	%
Na2O	sodium oxide	%
K2O	potasium oxide	%
P2O5	phosphorus pentoxide	%
Cr2O3	chromium oxide	%
NiO	nickel oxide	%
Topo-climatic variables		
Abbreviation	variables	Unit
Topography		
ddeg0	annual degree day above 0 °C	days * °C * 10
slope	slope steepness	°
mmind68	monthly moisture index	(1/10 mm) / months
sumradyy	shortwave raditaion per month	KJ/day
taveyy	monthly average temperature	°C * 100
topos	topographic position	
asp25	orientation of the slope	°
aspval	sinus of the orientation of the slope	%
swb	site water balance	(1/10 mm) / year
twi25	topographic wetness index	
sfroyy	number of frost day during the growing season	days * 100
pday	number of precipitation days per growing season	days
mnt25	elevation from digital model	m
precyy	monthly mean precipitation sum	mm
climate		
bio01	annual mean temperature	°C * 10
bio02	mean diurnal range (mean of monthly (max temp - min temp))	°C * 10
bio03	isothermality (bio02/bio07) (*100)	°C * 10
bio04	temperature seasonality (standard deviation ×100)	°C * 10
bio05	max temperature of warmest month	°C * 10
bio06	min temperature of coldest month	°C * 10
bio07	temperature annual range (bio05-bio06)	°C * 10
bio08	mean temperature of wettest quarter	°C * 10
bio09	mean temperature of driest quarter	°C * 10
bio10	mean temperature of warmest quarter	°C * 10
bio11	mean temperature of coldest quarter	°C * 10
bio12	annual precipitation	kg / m^2
bio13	precipitation of wettest month	kg / m^2
bio14	precipitation of driest month	kg / m^2
bio15	precipitation seasonality (coefficient of variation)	kg / m^2
bio16	precipitation of wettest quarter	kg / m^2
bio17	precipitation of driest quarter	kg / m^2
bio18	precipitation of warmest quarter	kg / m^2
bio18	precipitation of coldest quarter	kg / m^2

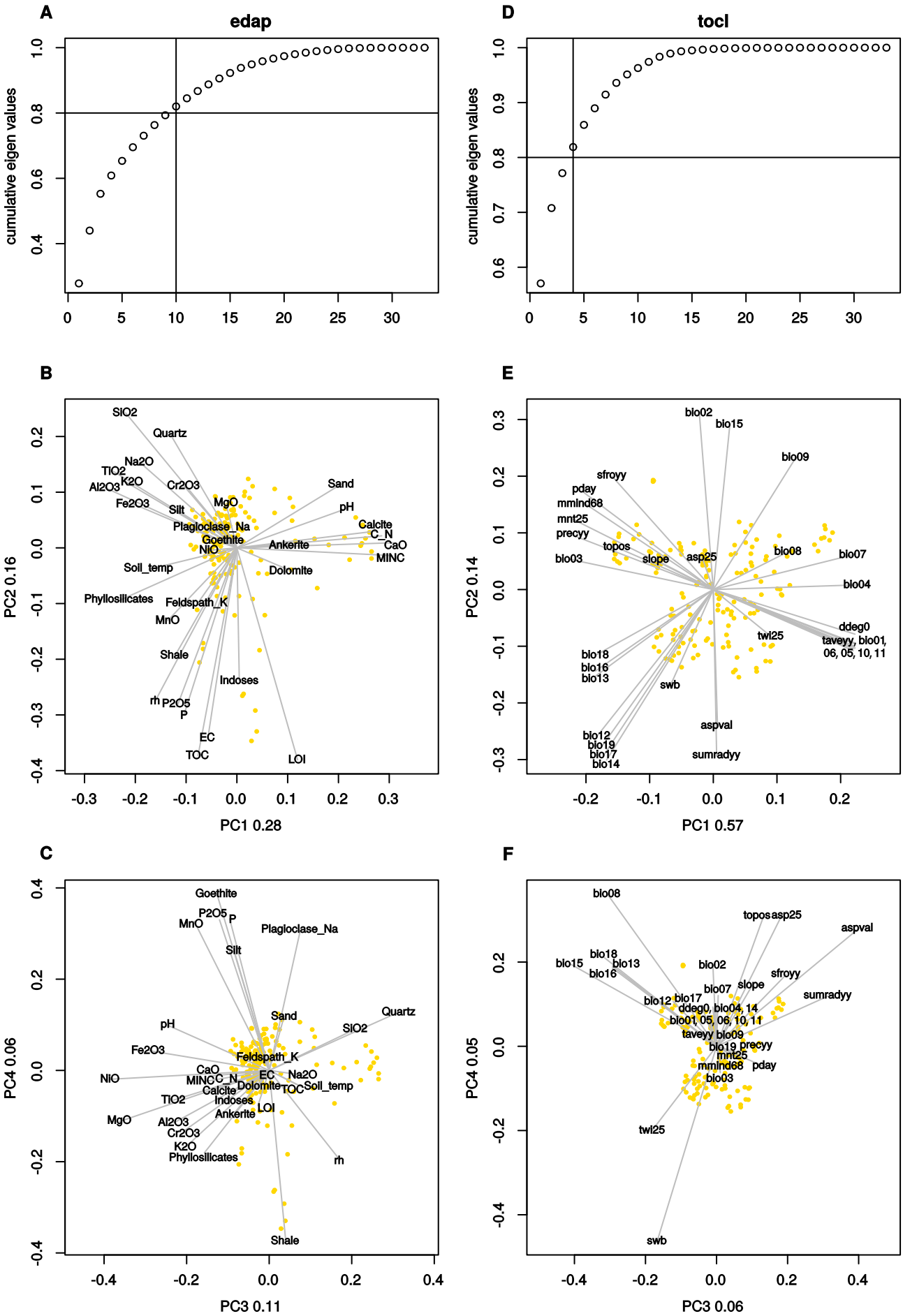

Figure S2: Principal component analysis (PCA) of the edaphic and topo-climatic descriptors measured from 162 meadow soil samples in the Swiss western Alps. The top row (A, D) shows cumulative eigenvalues of both PCA while the second (B, E) and third (C, F) sub-figures show the four first PCA axes.

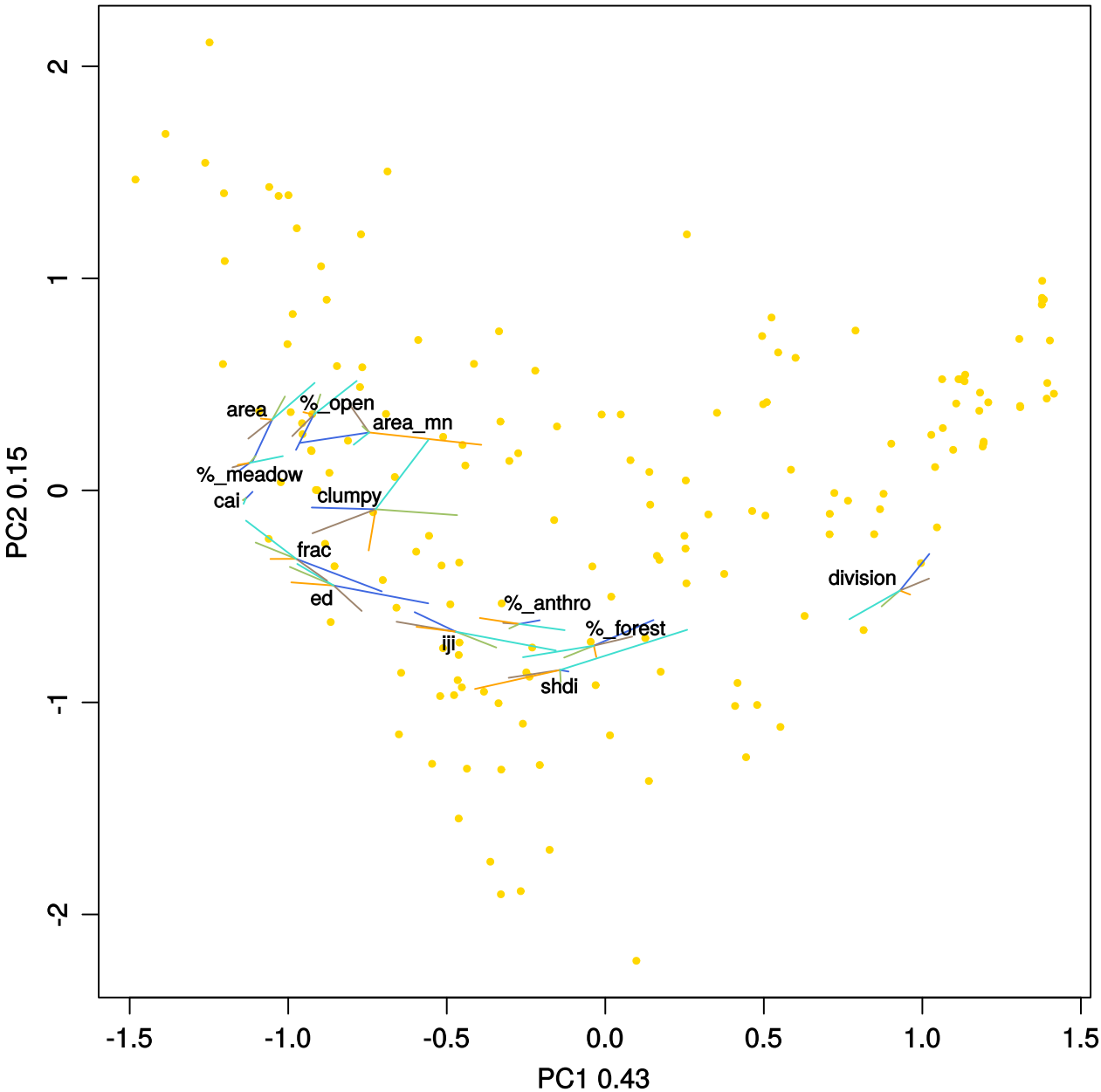

Figure S3: Principal component analysis (PCA) of 13 landscape descriptors measured around 162 meadows in the Swiss western Alps. Each landscape descriptor has been calculated in different buffers: radius of 100 (blue), 200 (brown), 500 (orange), 1000 (green) and 2000 (cyan) metres around the sampling plots.

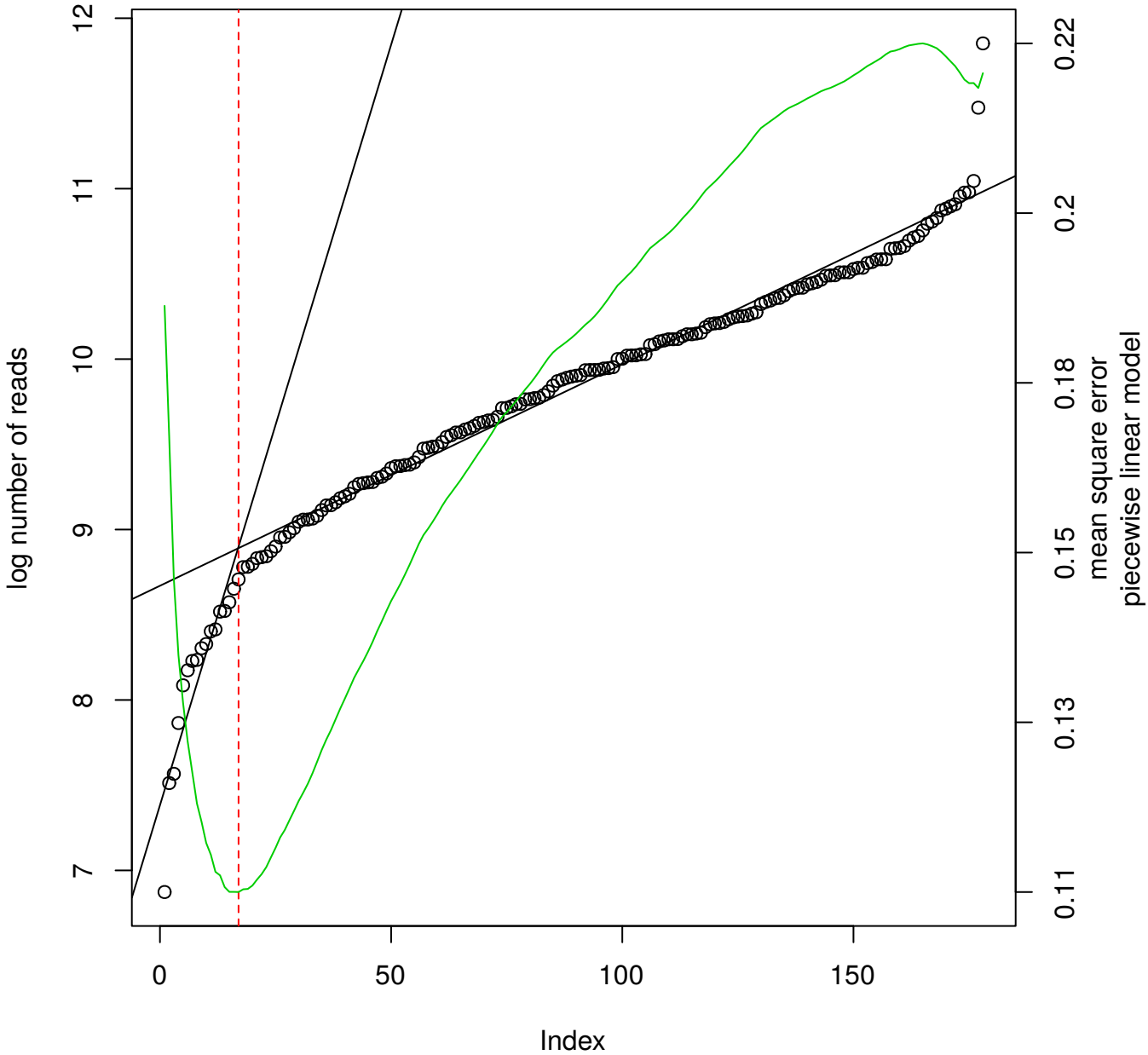

Figure S4: Determination of the threshold to remove samples with too few reads to be included in the analyses by piecewise linear model on the log of the number of reads sorted increasingly. The two black lines represent the two linear models at the optimal breaking point (vertical red line). The green line represents the mean square error for each breaking point. The 162 samples on the right of the breaking point were kept for further analyses.

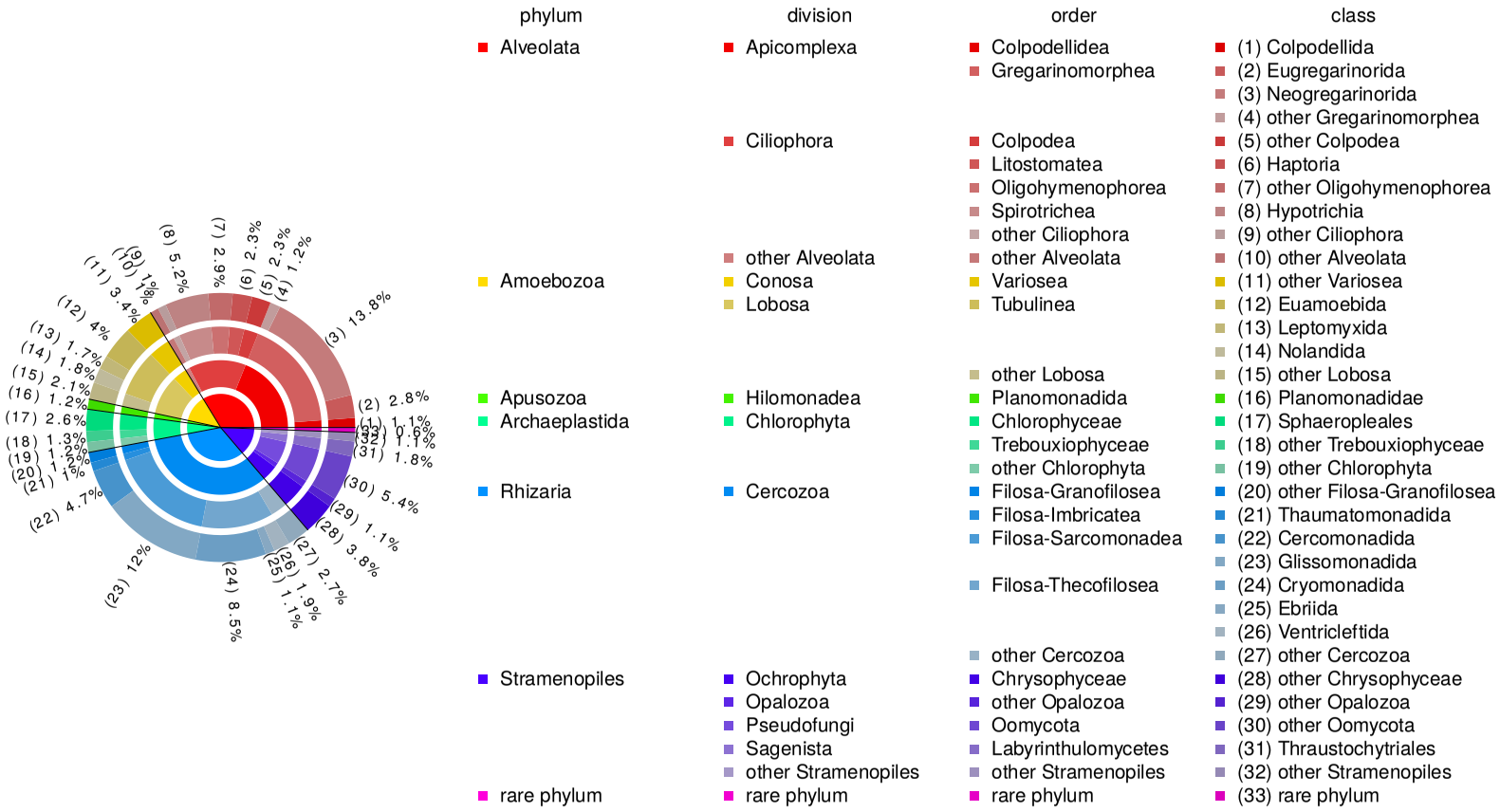

Figure S5: Protist taxa's relative abundance retrieved from 162 meadow soil sampled in the Swiss western Alps (3992 OTUs, 3334390 sequences). The different concentric circles represent the different taxonomic levels, phylum are toward the centre whereas classes are at the exterior of the pie chart. Taxa representing less than 1% of the community were pooled in the unidentified members of their lower taxonomic level.

Table S2: Contribution of the edaphic descriptors measured from 162 meadow soils in the Swiss western Alps to the ten first axes of a principal component analysis. The contributions for each axis were calculated as the relative contribution of the loadings values multiplied by 100. The five highest values are shown in bold.

		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Variance explained		27.82	16.18	11.26	5.59	4.48	4.18	3.53	3.26	3.00	2.70
	Soil_temp	0.96	0.14	0.16	0.04	0.07	**0.31**	0.15	0.10	0.04	**0.22**
	pH	1.20	0.26	**0.58**	0.13	0.01	0.07	0.06	0.02	0.12	0.08
	EC	0.32	**1.25**	0.01	0.02	0.03	0.04	0.04	0.06	0.16	0.14
	P	0.58	**1.10**	0.23	**0.44**	0.03	0.04	0.16	0.10	0.03	0.01
	TOC	0.42	**1.37**	0.19	0.05	0.05	0.07	0.11	0.06	0.04	0.03
	MINC	**1.69**	0.05	0.36	0.03	0.04	0.05	0.05	0.01	0.01	0.06
	C_N	**1.58**	0.08	0.23	0.04	0.03	0.03	0.06	0.05	0.07	0.06
	Sand	1.14	0.42	0.09	0.16	**0.42**	0.16	0.06	0.13	**0.20**	0.06
	Silt	0.65	0.25	0.20	0.34	0.23	**0.46**	**0.24**	0.15	0.15	0.12
	Shale	0.67	0.71	0.10	**0.49**	**0.24**	**0.26**	0.16	0.02	0.06	0.03
	rh	0.89	1.00	0.41	0.26	0.10	0.03	0.00	0.10	0.01	0.04
	Phyllosilicates	1.28	0.35	0.37	0.24	0.05	0.12	0.02	0.09	**0.18**	0.07
	Quartz	0.73	0.75	**0.75**	0.17	0.12	0.17	0.06	0.02	0.00	0.06
	Feldspath_K	0.43	0.37	0.01	0.03	0.08	0.03	0.06	**0.46**	**0.39**	0.04
	Plagioclase_Na	0.27	0.13	0.18	**0.40**	0.23	0.16	**0.32**	**0.42**	0.04	0.04
XRD	Calcite	**1.52**	0.11	0.28	0.05	**0.24**	0.04	0.08	0.07	0.07	0.13
	Dolomite	0.60	0.15	0.07	0.05	**0.61**	0.03	**0.17**	0.06	**0.18**	0.06
	Goethite	0.14	0.03	0.30	**0.51**	0.17	0.15	**0.29**	0.05	0.02	**0.30**
	Ankerite	0.57	0.02	0.19	0.12	**0.31**	**0.23**	0.16	0.04	**0.26**	**0.33**
	Indoses	0.03	0.88	0.20	0.06	**0.44**	0.06	0.16	**0.17**	**0.20**	0.04
	SiO2	1.21	0.89	0.50	0.12	0.01	0.07	0.01	0.00	0.03	0.03
	TiO2	1.34	0.50	0.55	0.09	0.04	0.09	0.06	0.03	0.05	0.02
	Al2O3	**1.44**	0.40	0.55	0.15	0.11	0.06	0.04	0.07	0.07	0.02
	Fe2O3	1.13	0.29	**0.69**	0.06	0.01	0.18	0.12	0.03	0.01	0.02
	MnO	0.76	0.48	0.43	**0.43**	0.08	0.14	**0.17**	0.04	0.08	**0.18**
	MgO	0.12	0.29	**0.86**	0.15	0.10	**0.23**	0.04	0.02	0.02	**0.20**
XRF	CaO	**1.71**	0.03	0.35	0.02	0.03	0.02	0.06	0.02	0.01	0.05
	Na2O	1.06	0.57	0.20	0.01	0.20	0.10	**0.27**	0.05	0.12	0.04
	K2O	1.21	0.43	0.48	0.22	0.19	0.18	0.12	0.15	0.11	0.05
	P2O5	0.65	**1.05**	0.29	**0.43**	0.01	0.06	**0.17**	0.11	0.03	0.00
	LOI	0.66	**1.40**	0.06	0.10	0.00	0.06	0.05	0.04	0.04	0.00
	Cr2O3	0.58	0.41	0.49	0.17	0.03	**0.32**	0.00	**0.30**	0.15	0.05
	NiO	0.30	0.02	**0.91**	0.03	0.18	0.14	0.00	**0.23**	0.01	0.13

Table S3: Contribution of the topo-climatic descriptors measured from 162 meadow soils in the Swiss western Alps to the four first axes of a principal component analysis. The contributions for each axis were calculated as the relative contribution of the loading values multiplied by 100. The five highest values are shown in bold.

		PC1	PC2	PC3	PC4
Variance explained		57.05	13.73	6.36	4.76
	ddeg0	**2.44**	0.22	0.01	0.09
	slope	0.98	0.14	0.16	0.15
	mmind68	2.28	0.43	0.02	0.04
	sumradyy	0.06	**0.81**	**0.50**	0.13
	taveyy	**2.44**	0.29	0.07	0.03
	topos	1.65	0.20	0.21	**0.33**
	asp25	0.19	0.15	0.29	**0.33**
	aspval	0.07	0.67	**0.64**	**0.29**
	swb	0.74	0.47	0.27	**0.49**
	twi25	0.99	0.23	0.29	0.21
	sfroyy	1.74	0.55	0.32	0.18
	pday	2.22	0.47	0.22	0.05
	mnt25	**2.41**	0.34	0.07	0.02
	precyy	**2.41**	0.26	0.10	0.02
Bioclimatic variables	bio01	**2.42**	0.28	0.03	0.07
	bio02	0.24	**0.86**	0.02	0.20
	bio03	**2.48**	0.15	0.02	0.07
	bio04	**2.48**	0.02	0.00	0.10
	bio05	**2.43**	0.26	0.03	0.07
	bio06	**2.41**	0.30	0.03	0.07
	bio07	**2.41**	0.17	0.01	0.13
	bio08	1.29	0.19	**0.50**	**0.38**
	bio09	1.42	0.64	0.05	0.03
	bio10	**2.43**	0.26	0.03	0.07
	bio11	**2.41**	0.29	0.03	0.07
	bio12	2.00	**0.72**	0.20	0.11
	bio13	2.02	0.42	**0.47**	0.20
	bio14	1.84	**0.83**	0.02	0.10
	bio15	0.29	**0.81**	**0.66**	0.21
	bio16	2.03	0.40	**0.47**	0.20
	bio17	1.88	**0.81**	0.07	0.10
	bio18	2.02	0.32	**0.51**	**0.22**
	bio19	1.92	**0.76**	0.03	0.00

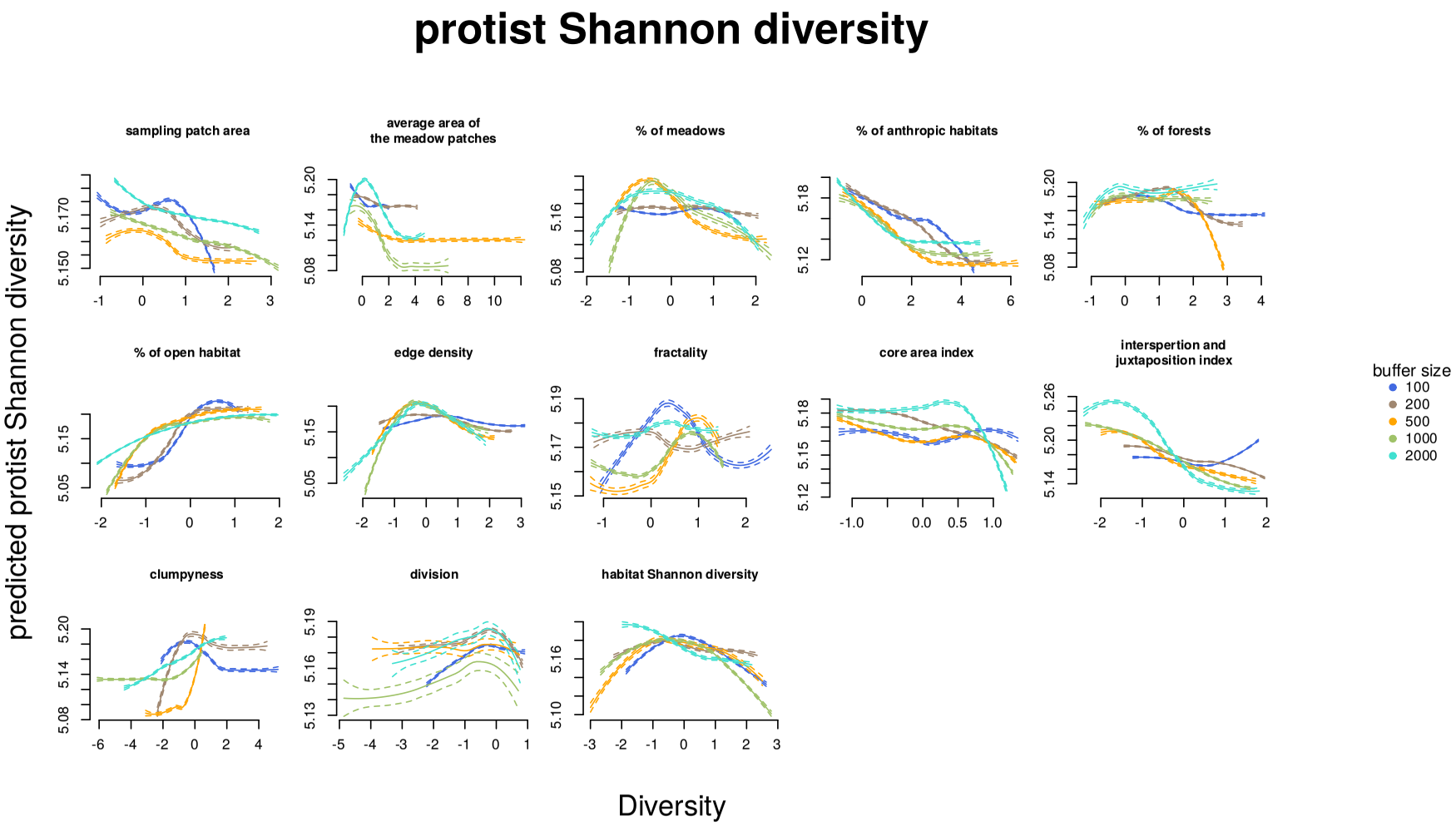

Figure S6: Effect of landscape descriptors on protist diversity. Partial dependence plots showing the overall protist diversity response in function of 15 landscape descriptors from meadow soils taken in the Swiss western Alps.

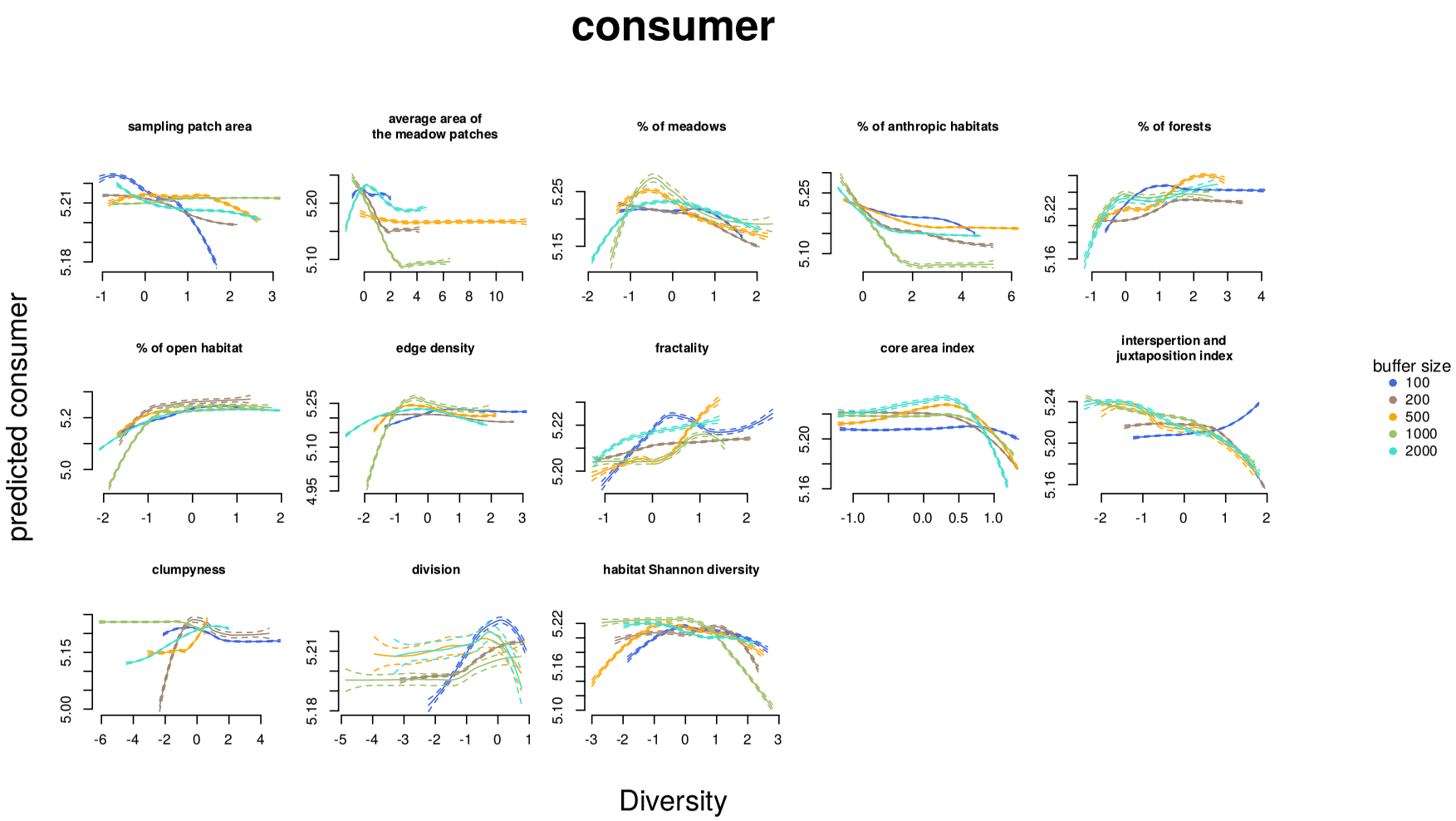

Figure S7: Effect of landscape descriptors on consumer protist diversity. Partial dependence plots showing the overall protist diversity response in function of 15 landscape descriptors from meadow soils taken in the Swiss western Alps.

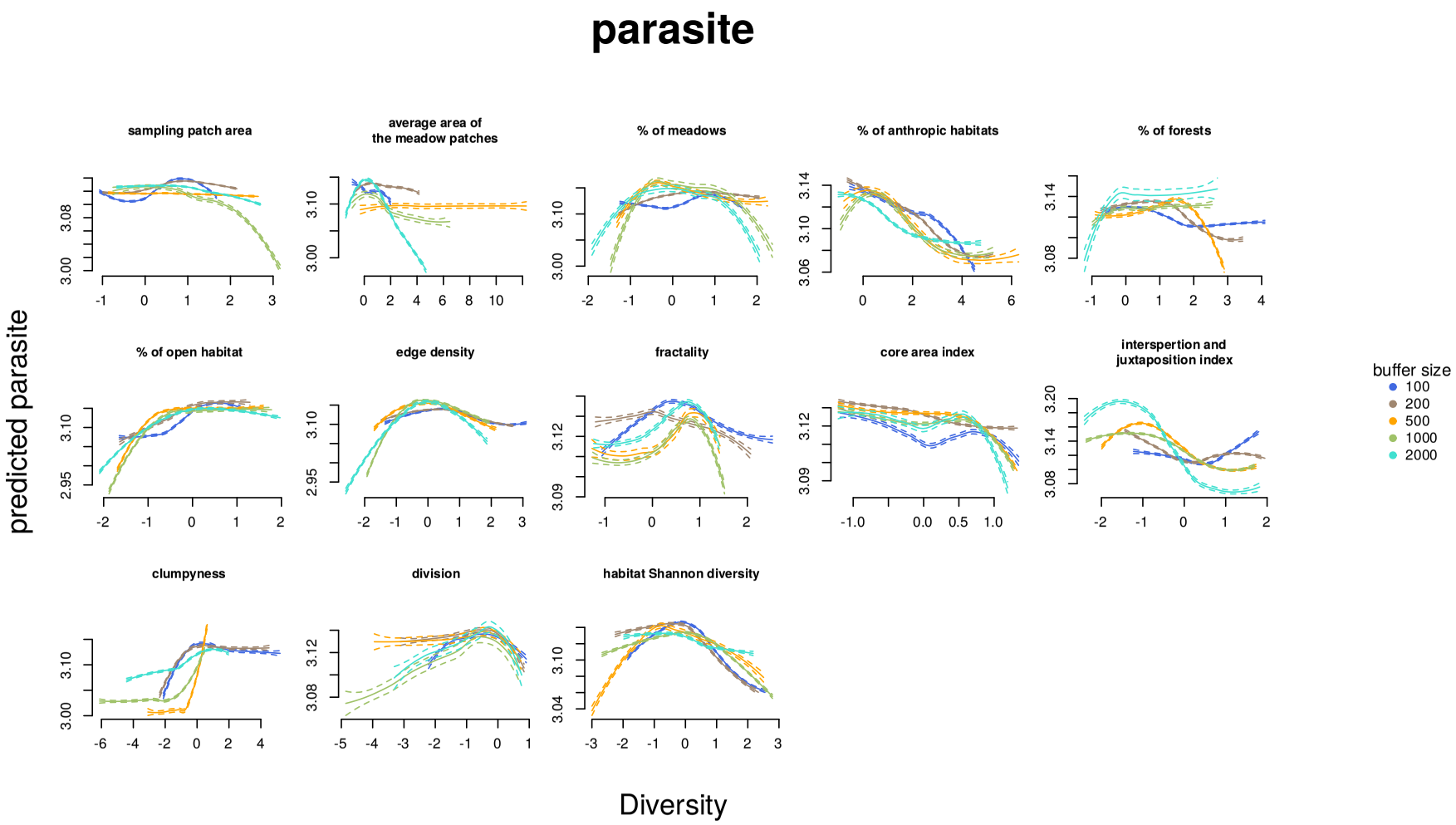

Figure S8: Effect of landscape descriptors on parasite protist diversity. Partial dependence plots showing the overall protist diversity response in function of 15 landscape descriptors from meadow soils taken in the Swiss western Alps.

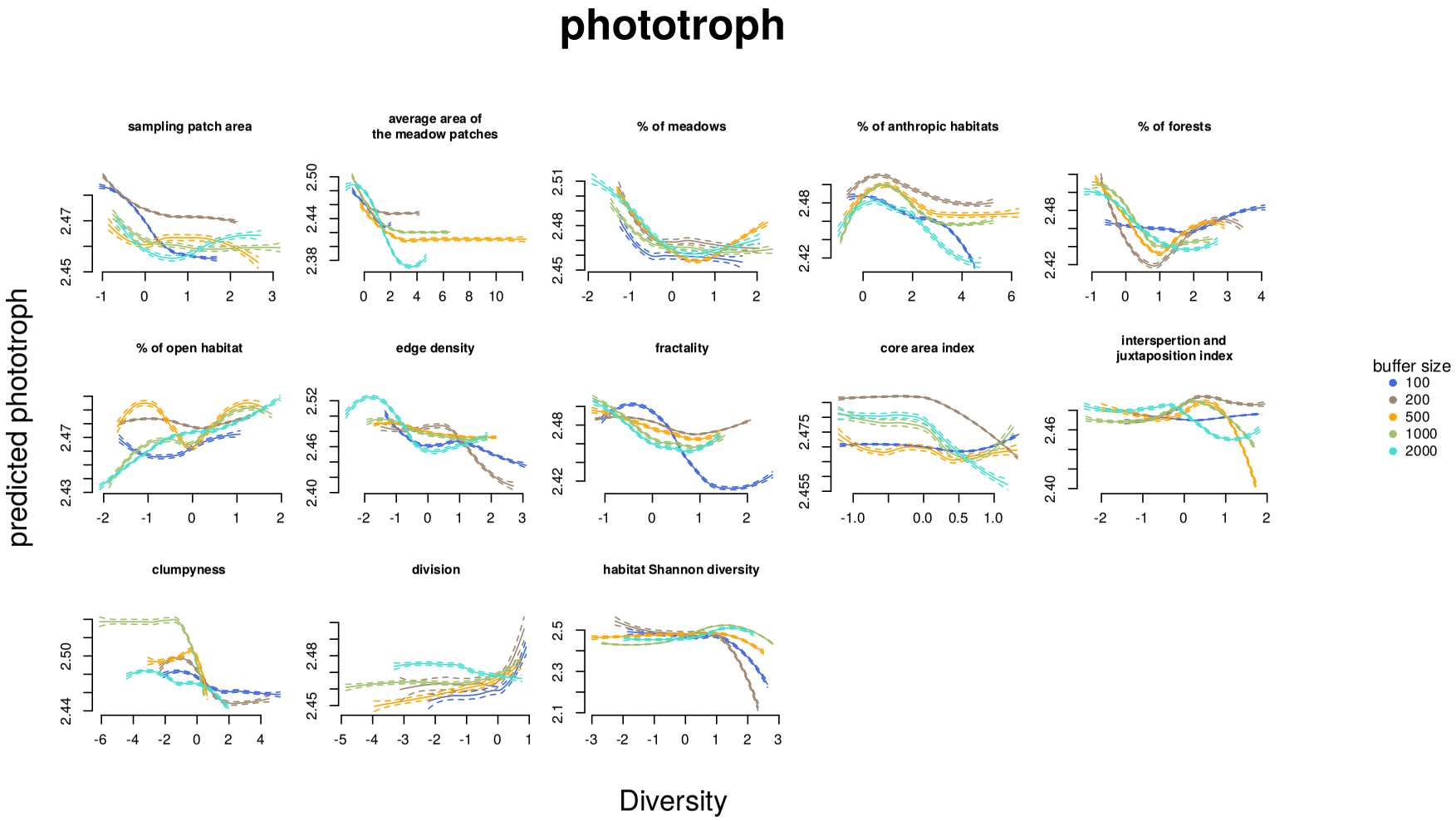

Figure S9: Effect of landscape descriptors on phototrophic protist diversity. Partial dependence plots showing the overall protist diversity response in function of 15 landscape descriptors from meadow soils taken in the Swiss western Alps.

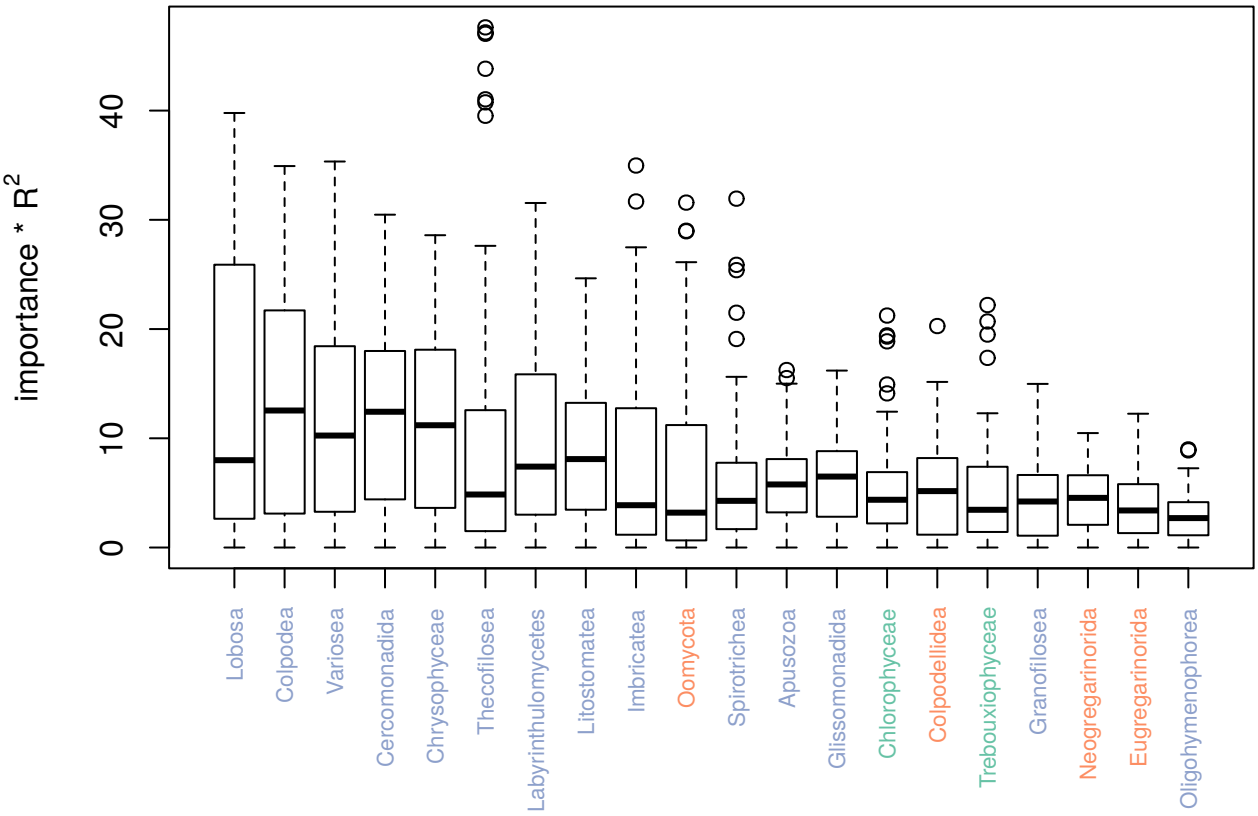

Figure S10: Importances of landscape metrics in explaining the diversity of dominant soil protist taxa in the Swiss western Alps. The functional group of each taxon is represented by the taxon's color. Blue: consumers, green: phototrophs, orange: parasites. All importance metrics were weighted by the R^2 of their model to ensure comparability. Each boxplot takes into account the models computed for the 15 landscape metrics and the 5 spatial buffers.