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Master's thesis in Geoinformatics for Urbanised Society

Role of soil properties and landscape composition in arbuscular mycorrhizal fungi communities of Estonia

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Tartu 2021

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Mulla omaduste ja maastikustruktuuri mõju Eesti krohmseenekooslustele

Abstrakt

Krohm- ehk arbuskulaarmükoriissete (AM) seenekoosluste koosseis ja liigirikkus varieeruvad ökosüsteemide lõikes. Erinevusi põhjustavad nii biootilised kui abiootilised tegurid, muuhulgas inimtegevus, samas on suhteliselt vähe teada maastikustruktuuri ja -muutuste mõjust. Käesolev töö uurib 24 prooviala näitel krohmseenekoosluste liigirikkuse ja koosseisu seost mullaparameetrite ning maastikustruktuuri mõõdikutega. Leiti, et krohmseente liigirikkus on suurem mitmekesise maastiku ning tüsedama orgaanilise horisondi korral, mullastruktuuri mõju ei tuvastatud. Krohmseenekoosluste koosseisu mõjutavate tegurite väljaselgitamiseks kasutati üldistatud erinevusanalüüsi (GDM). Leiti, et koosluse koosseisu mõjutavad tähtsuse järjekorras kooslustevaheline geograafiline kaugus, mullastruktuur ning maastiku mitmekesisus.

Märksõnad: Glomeromycotina, maastikumõõdikud, mullastruktuur, üldistatud erinevusanalüüs

CERCS-i kood: P510, B230, B270

Role of properties and landscape composition in arbuscular mycorrhizal fungi communities of Estonia

Abstract

Arbuscular mycorrhizal (AM) fungal communities and richness vary across ecosystems. The variation is the result of biotic and abiotic forces, alongside anthropogenic activities, yet the role of the landscape configuration and changes has been poorly studied. Here, the relation AM fungal richness and community turnover with soil and landscape configuration metrics and change were tested for 24 sites in Estonia. AM fungal richness was positively related to the landscape diversity and the depth of the topsoil layer. No significant relations were found with soil texture variables. A generalized dissimilarity model was used to identify the factors responsible for community turnover. Turnover in community composition was explained by three factors, in order of importance: geographical distance between communities, soil texture, and change in the landscape diversity.

Keywords: *Glomeromycotina*, landscape metrics, soil texture, generalized dissimilarity model

CERCS Code: P510, B230, B270

1. INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are a group of symbiotic organisms associated with ~70% of vascular plants (Brundrett & Tedersoo, 2018). The arbuscular mycorrhizal symbiosis provides benefits such as improved plant mineral nutrient acquisition and resistance to abiotic and biotic stress, influencing the plant and soil communities (Tedersoo *et al.*, 2020).

The majority of taxa from this group is widely distributed across the world. Nevertheless, the richness, abundance, and community composition of AM fungi vary across ecosystems (Davison *et al.*, 2015). Patterns of AM fungi communities have been studied at different scales. At global scale, differences between global ecosystems given by historical changes, spatial and environmental variation (Pärtel *et al.*, 2017). At the regional scale spatial variation in soil properties and geographical distance between sites are the main factors driving the community assembly (Jansa *et al.*, 2014; Xu et al., 2016). Besides soil properties, other factors such as climate (Chen *et al.*, 2017), host traits (Chagnon *et al.*, 2015) historical changes of the plant communities (Bittebiere *et al.*, 2020) are recognized factors driving the AM fungi communities.

Anthropogenic modifications of the ecosystems have an impact on biological communities. For AM fungi communities, the intensity of land use and changes in environmental factors derived from it change the AM fungi community composition (Sepp et al., 2018). Since this symbiotic interaction plays key roles in plant diversity and nutrient cycling, it is essential to know the factors driving the AM fungi community composition and diversity and how anthropogenic modification of ecosystems affects them.

For AM fungi, only a few studies have dealt with landscape perspective, and the results of the impact of change in the landscape are contrasting. AM fungi spore richness was found positively related with an increase in forest patch area (Grilli *et al.*, 2012). Nevertheless, using molecular methods, AM fungal taxa richness and community composition did not differ in relation with forest patch area (Grilli *et al.* 2015). More studies with a comparable AM fungi species delimitation should provide a wider view of the importance of the landscape structure and change of it in AM fungi richness and communities.

The current study aimed to investigate the role of soil properties, landscape configuration, and the historical changes of the landscape in the AM fungi richness and community compostion by combining spatial analysis and community ecology techniques.

2. THEORETICAL OVERVIEW

2.1 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal (AM) fungi, from the subphylum Glomeromycotina (Spatafora *et al.*, 2016), are an ancient group of microscopic symbionts that involves 342 (http://www.amf-phylogeny.com) morphological defined taxa or ~500 to ~1000 molecular defined taxa (Kivlin *et al.*, 2011; Öpik *et al.*, 2014). The symbiosis between plant roots and AM fungi has been estimated to be presented in 71% of vascular plants (Brundrett & Tedersoo, 2018). AM fungi are characterized by the formation of intraaradical hyphal structures, such as coils, vesicles, and arbuscules which form an interface for nutrient exchange. Intrradical mycelium is connected to extraradical mycelium, which was the function of nutrient uptake, colonization of roots, and spore formation (Smith & Read, 2008). Other characteristics of AM fungi are the production of coenocytic hyphae and multinucleated asexual spores. Besides that, evidence of sexual reproduction has only been found in strains of one species (Ropars *et al.*, 2016).

AM fungi provide nutrients to their plant partners in exchange for carbon (Smith & Read, 2008) and influence plant diversity by playing key roles in plant establishment, below-ground interactions, and stress tolerance (Tedersoo *et al.*, 2020). Colonization by AM fungi has a subsequential improvement in nutrient uptake, reflected in the plant concentration of macro and micro-nutrients. It promotes an increase in photosynthate production and biomass accumulation, especially in nutrient-deficient soils (Begum *et al.*, 2019).

AM fungi have a cosmopolitan distribution, being found in most terrestrial ecosystems and latitudes (Chaudhary *et al.*, 2008). The comprehension of AM fungi diversity and distribution at a local and global scale has increased due to the availability of DNA sequences and their classification in unified-comparable phylogroups (Öpik *et al.*, 2014; Tedersoo, 2017). Although AM fungi at a global scale have shown low endemism, community composition and richness vary with environmental variables (precipitation, soil organic carbon content, and pH) and the geographical distance between sampling sites across the world (Davison *et al.*, 2015). Three broad categories of forces that influence directly AM fungi ability to colonize and establish in a particular habitat were proposed by (Chaudhary *et al.*, 2008): abiotic external forces, such as precipitation and edaphic characteristics; external biotic forces, such as host plant identity and biotic interactions, and intrinsic properties of species (e.g., dispersal ability). Additionally, these three forces indirectly influence the AM fungi distribution by its

interaction, e.g., abiotic forces as climate determinate plant communities with which AM inhabit. The importance and effect of these forces in AM diversity and community composition have been studied in different ecosystems and spatial scales. Additionally, anthropogenic disturbances have also been taken into account to understand the current distribution of AM fungi.

2.2 Abiotic drivers of AM fungi communities

Abiotic external factors can directly influence the AM fungal communities and species distribution. Soil physical and chemical properties have received particular attention since AM fungi extraradical mycelium spreads in the soil matrix.

Soil pH has an essential role in AM community composition, is also directly related to other soil properties as C/N ratio and P concentration (Dumbrell *et al.*, 2010). Soil pH affects mycelium growth, spore production (Yang *et al.*, 2011), and nutrient acquisition since it controls the availability of soil nutrients (Fitzsimons *et al.*, 2008). AM fungal species respond differently to soil pH, increasing the abundance of some taxa at specific ranges (Jansa *et al.*, 2014) and influencing root colonization (Klichowska *et al.*, 2019).

Physical properties of the soil also play a role in AM fungi community composition. The latter is affected by soil water capacity, bulk density, and percentage of coarse fragments in the soils and affects root colonization. However, its effect is lower than other soil properties as N content (Klichowska *et al.*, 2019).

The availability of soil nutrients as P and N has also been proved to influence AM fungi communities. An increase of P thought fertilization has a negative effect on AM fungi taxa richness, and influences community composition (Camenzind *et al.*, 2014). Similarly, contrasting levels of soil N content influence AM fungal communities, but its effect on AM fungal richness is still not apparent, showing different patterns of response (Lu et al., 2020).

The listed soil proprieties can explain a large part of the variation in AM fungal communities, and a similar proportion of variation can be attributed to climate variables (Chen *et al.*, 2017; Klichowska *et al.*, 2019). Temporal variation in temperature alters the AM fungal community composition. Yang *et al*. (2013) reported an effect of three years of warming on AM fungi communities. Similar results were obtained by Zhang T *et al*. (2016) after 5 years of warming.

AM fungi vary in their ability to tolerate drought and water availability. AM fungal richness and abundance decrease when precipitation increases (Chen *et al.*, 2017). As with the previously listed variables, precipitation also influences soil properties and vegetation. Compared with soil properties and geographical distance, climatic factors have a small contribution to AM community assembly.

At global and large scales, geographical distance significantly influences AM fungal communities (Davison et al., 2015; Xu et al., 2016). At more minor scales, geographical distance can also explain variation in AM fungal communities, but its contribution gets lower, being soil properties the main driver (Huang *et al.*, 2019). Geographical distance reflects differences in climate, soil type, and land use, etc. Along with soil properties, geographical distance is the main factor driving AM fungal communities (Jansa *et al.*, 2014), followed by climate and elevation, which cannot explain the total variation of AM fungal communities across the world (Kivlin *et al.*, 2011).

2.3 Biotic drivers of AM fungi communities

AM fungi are plant symbionts (Brundrett & Tedersoo, 2018). Therefore, their presence and development in ecosystems are determined by their interaction with plants, acting as biotic factors. The AM symbiosis is generally pointed out to be generalist, establishing the mycorrhizal symbiosis of different taxonomical groups, even simultaneously (van Geel *et al.*, 2018). However, plant communities have differential responses to distinct AM fungi communities (Klironomos, 2003). Partner selection by plants and fungi based on species nutrient acquisition strategies has been observed (Kiers *et al.*, 2011).

At local scale, where there is a low variation on environmental conditions, plant traits and identity play key roles in the AM fungal communities. Plants with similar traits in C and soil nutrient acquisition host similar AM fungal communities with phylogenetically related taxa (Chagnon *et al.*, 2015). This pattern was also observed in traits as growth form, herbaceous or semi-woody, and life cycle, annual or perennial (López-García *et al.*, 2017), and in plants with a similar functional group, forbs or grasses, been the AM fungal taxa richness also group dependent (Šmilauer *et al.*, 2020). The shared history between fungal and plant partners is another determinant factor of root AM fungal community composition, contrasting between exotic and native plants (Klironomos, 2003). These studies indicate the importance of plant host identity, taxonomical or functional, in AM fungi communities. The response of plant

communities to the environment and their presence in ecosystems needs to be also considered a driver of AM fungi communities since they can exhibit functional reactions to it (Xu *et al.*, 2020). And it should also notice that plant communities and richness are also influenced by AM fungi communities (Toussaint *et al.*, 2020).

2.4 Land use as a driver of AM fungi communities

Anthropogenic activities modify biological communities by changing the distribution and the abundance and richness of biological groups and modifying ecosystems (Boivin *et al.*, 2016).

In forest and grassland habitats, the community composition of AM fungi communities responds to land-use intensity. Sepp *et* al. (2018) found contrasting communities under different land-use intensities. This indicates that land-use change should be considered while studying its effect on AM fungi.

In agricultural sites, AM fungal communities have shown differences given by land-use history, crop rotation techniques, and inorganic fertilizers, reflecting the impact of human activities in the ecosystems (Ontivero *et al.*, 2020). The anthropogenic modifications to the environment do not only give the resultant AM fungal communities in agricultural fields. Still, they are also given by the history of the sites, as previous land use and environmental conditions (Faggioli *et al.*, 2019).

2.5 Landscape structure

Spatial and temporal environmental heterogeneity influence AM fungal communities, exhibiting horizontal spatial autocorrelation at small scales (6-9 m) and vertical soil variation of communities in forest ecosystem soil (Bahram *et al.*, 2015).

Biodiversity of micro-organisms have been related to the composition of their habitat (landscape structure). In micro-organisms plant communities' present and past spatial structure (plant landscape) modulate the AM fungi community assembly (Bittebiere *et al.*, 2020).

Studies were landscape ecology principles as fragmentation and heterogeneity in the study of micro-organisms biodiversity and community composition at large scales are sparse due to gaps in knowledge of micro-organisms habitat requirements and challenges in the observation and taxonomical identification of them (Mony *et al.*, 2020).

As AM fungi are mutualistic organisms that depend on the host presence to develop, and changes in the landscape might also reflect a difference in the soil properties, landscape fragmentation should be considered to understand the distribution of these organisms.

For AM fungi, landscape fragmentation, specifically decrease in forest fragment size, has shown different patterns. Mainland and insular forest loss and fragmentation were found to not affect the AM fungal morphospecies richness, and that AM fungal communities of distant patches with the same vegetation type are more similar than close patches with different vegetation (Mangan *et al.*, 2004). Contrary to this, the size of patches of forest immersed in an agricultural matrix was found not only positively correlated with the abundance and richness of AM fungi morphospecies but also influencing the AM colonization, increasing with the size of the forest area (Grilli *et al.*, 2012). Using a molecular approach, Grilli *et al.* (2015) found neither AM fungal richness nor AM fungal communities of a plant species were related with forest fragment size. However, the effect of habitat fragmentation in AM fungal richness and communities in the soil were not analysed.

Habitat isolation plays a crucial role in species distribution when dispersion is a limiting factor (Mony *et al.*, 2020). In the case of AM fungi, these have been shown to have a cosmopolitan distribution, suggesting that dispersal is not a limitation (Davison *et al.*, 2015) and being generalist, the presence of a specific partner is not a limiting issue.

3. DATA AND METHODOLOGY

All data processing and analysis were done using R (R Core Team, 2018) unless otherwise indicated. Data and analysis are available in: https://github.com/OscarZarateM/Role-of-soil-properties-and-landscape-composition-in-shaping-arbuscular-mycorrhizal-fungi-communitie

3.1 Data

3.1.1 Arbuscular mycorrhizal fungal data

Data from the Maarj*AM* database (Öpik *et al.*, 2010, https://maarjam.botany.ut.ee/) was chosen to explore the communities and diversity patterns of AM fungi. The database contains Glomeromycotina DNA sequence data from ecological studies and cultured fungi and associated metadata. DNA sequences are classified in phylogroups named Virtual Taxa (VT)

based on sequence similarity, which allows studying richness, diversity, and distribution of AM fungi and its relation with biotic and abiotic factors (Öpik *et al.*, 2010).

Records of AM fungi in Estonia were retrieved from the Maarj*AM* database in January 2020. The data was downloaded in xlsx format.

The downloaded table contained 42 columns with two identifiers in the original database (2), taxonomical information for the fungal sequence (6), information of the source of the sequence (13), information if the plant host when the source was plant roots (6), geographical information, country, state, location and GPS coordinates from the sample collection sites (5), information related to the sequencing method (6), and reference to the original publication were the sequences were reported (4). Records from cultures were filtered out.

The data contained 1347 records of sequences for Estonia, from 49 unique sites (Figure 1). The assigned VT of each record was used as the taxonomical unit to compare AM fungi richness and communities. For further community composition analysis only sites with more than 20 records were used.

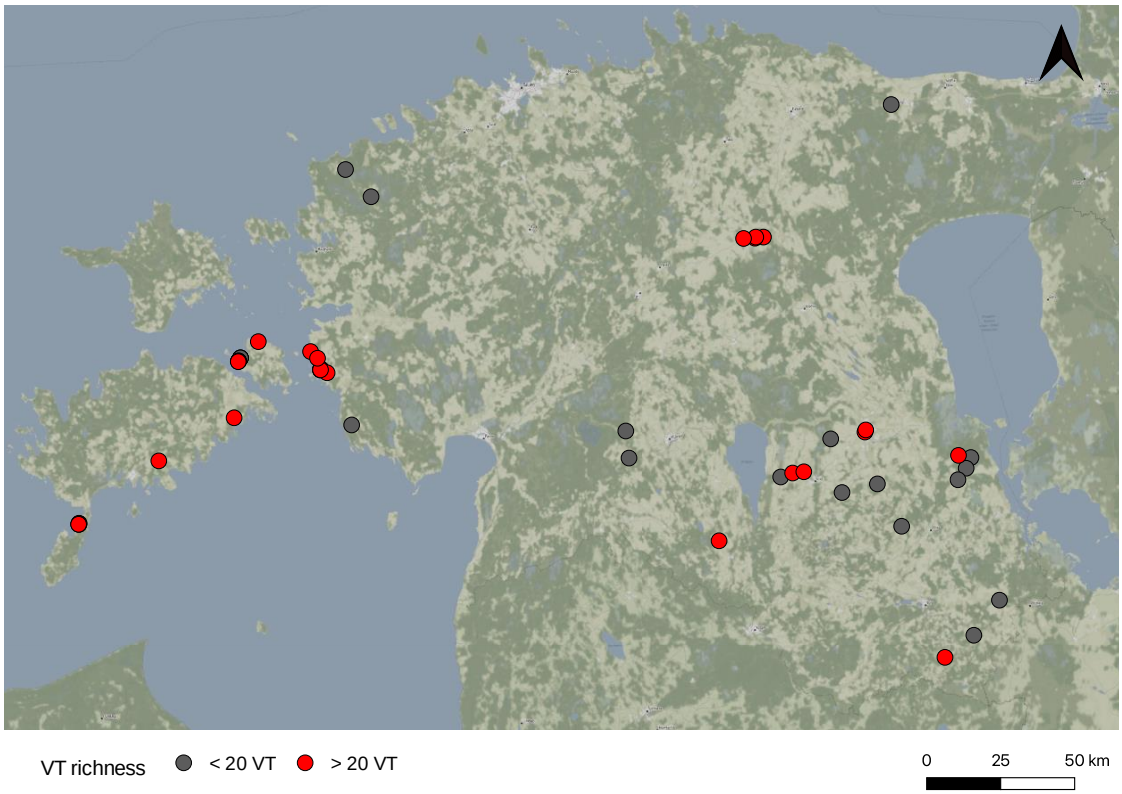

Figure 1. Map of sites with AM fungi records in Estonia from Maarj*AM* database.

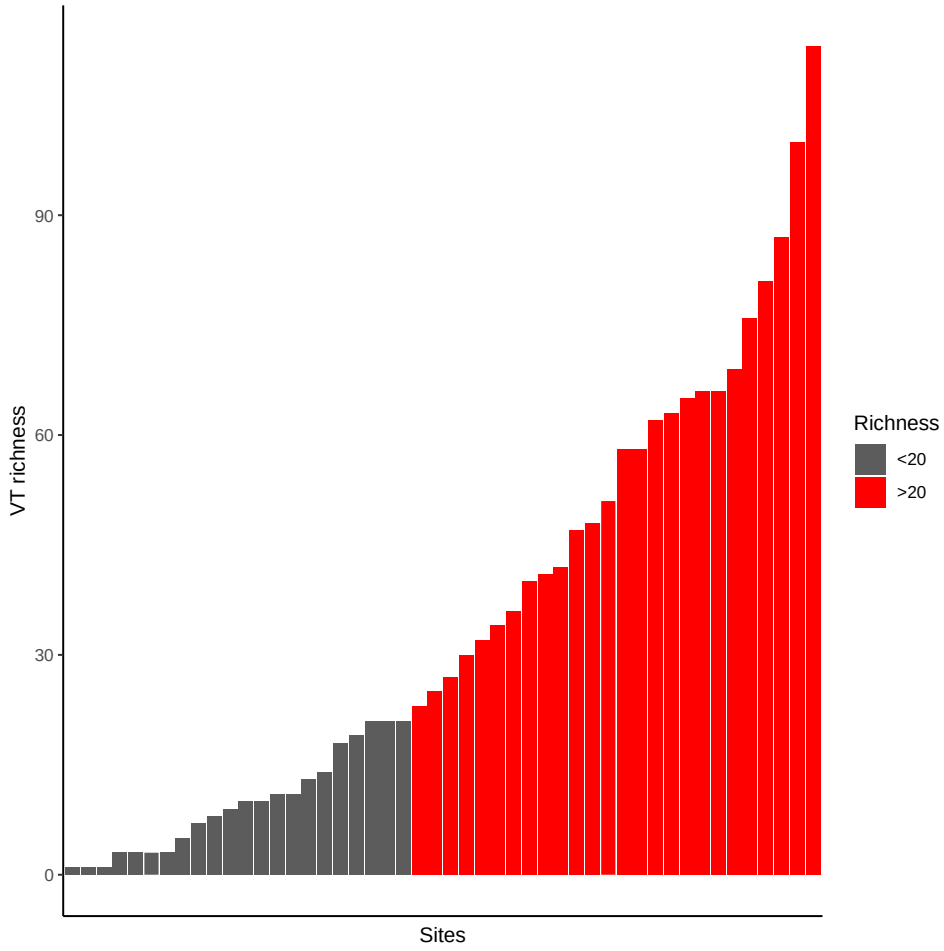

Figure 2. AM fungal taxon (VT) richness per site

3.1.2 Landscape data

To relate the AM fungi data with land use from where the sequences were collected, topographic data from Estonia was downloaded (December 2020) from the Estonian Topographic Database (ETAK) as shapefiles (https://geoportaal.maaamet.ee). Only layers indicated as land-use were selected, resulting in 11 shapefiles. Each file contained polygons of each for the 11 land-use types, and information regarding each polygon (e.g., id in ETAK, id in the layer, code of land use type). Table 1 shows the original names of the files. The 11 shapefiles were merged into a single dataset. To analyze the landscape where AM has been registered, 1000 m buffer zones were created around each site with AM fungi records. Topographic features inside each buffer zone were clipped and stored in a single shapefile for each site. Buffer creation and clipping were done using the package sf (Pebesma, 2018). The latter was used as an input to the ArcGIS extension Patch Analysis, to calculate landscape metrics for each site. Five metrics were selected to analyze the landscape diversity, patch size, density, and shape, with AM fungi (Table 2).

To register and later quantify the land-use change in each site with AM fungi records from the Maarj*AM* database, historical maps of Estonia were accessed via Web Map Service from the Estonian Land Board in QGIS v 3.10. In total 4 historical maps printed between 1894 and 2003 were revised (Table 3). Polygons inside each site 1000 m buffer zones representing the land use type were manually digitalized. The land-use type of the polygons was assigned following the types in the topographic dataset (Table 1) excluding watercourses and roads. A shapefile for each site in each revised WMS was produced and used landscape metrics were calculated using Patch Analysis.

Table 1. Topographic retrieved from ETAK and represented type in English.

Original Name	Type
E_201_meri_a	Sea
E_202_seisuveekogu_a	Lake
E_203_vooluveekogu_a	Watercourse
E_301_muu_kolvik_a	Wasteland/green area
E_302_ou_a	Yard
E_303_haritav_maa_a	Arable land
E_304_lage_a	Grassland
E_305_puittaimestik_a	Forest
E_306_margala_a	Swamp
E_307_turbavali_a	Peat_field
E_501_tee_a	Roads

Table 2. Landscape metrics from Patch Analysis

Name	Abbreviation	Metric type
Shannon's Diversity Index	SDI	Diversity
Mean perimeter area ratio	MPAR	Shape complexity
Area-Weighted Mean Shape Index	AWMSI	Shape complexity
Mean Patch Size	MPS	Edge
Number of Patches	NumP	Patch density and size

Table 3. WMS used from the Estonian Landboard.

Original Name	Printed Period
Üheverstased kaardid 1:42 000	1894-1922
Eesti Vabariigi topograafilised kaardid 1:50 000	1923-1939
Eesti baaskaart 1:50 000	1994-1998
Eesti baaskaart 1:50 000	1997-2003

3.1.3 Soil Data

To know the soil type and variables in which sites were registered, the EstSoil-EH dataset was used (Kmoch *et al*, 2021). The downloaded dataset consisted of a vector dataset that maps more than 750 000 soil units throughout Estonia at a scale of 1:10 000. For each soil unit, it describes the soil type, quality, texture, and physical variables that might influence AM fungi communities and diversity.

The dataset was downloaded in December 2020 as a shapefile. Table 4 shows the variables of interest from the soil dataset.

Table 4. Selected variables from the Est-Soil-EH dataset

Name of variable	Description
Z1	depth in mm of soil layer
SAND1	Sand % mass of fine earth fraction
SILT 1	Silt % mass of fine earth fraction
CLAY1	Clay % mass of fine earth fraction
ROCK1	Rock fragment content% volumetric
SOC1	Organic C % soil weight
BD1	Bulk density g/cm³
AWC 1	Available water capacity mm H_2O/mm soil

To relate the soil data with the sites with AM fungi, the dataset with the points of the latest were projected to EPSG 3301 to match the former and a spatial join was performed with the sf package (Pebesma, 2018). Only soil variables values of the first layer were used

3.2 Methodology

Landscape change in time

In the interest of analyzing the effect of landscape change in time with the community composition of AM fungi the historical digitalized landscapes were used. Landscape dissimilarity in terms of land use type areas was calculated using Euclidean distance between subsequential times: 1894-1922 and 1923-1939, 1923-1939 and 1994-1998, 1994-1998 and 1997-2003; 1997-2003 and 2021. The total dissimilarity was calculated as the sum of distances in each time frame and used as an indicator of landscape change. The greater the value the more the landscape has changed in time.

Change in SDI was calculated as the sum of differences in SDI between each time frame. Positive values indicated an increase in SDI and negative values a decrease in SDI. The variable change in SDI was coded as binary to represent a decrease or increase in SDI.

Community analysis

The goal of this study was to analyze the effect of geographical distance, landscape configuration, landscape change, and soil variables in shaping the community composition of AM fungi. First, soil and landscape configuration variables were analyzed to remove highly correlated variables. The correlation was tested using Spearman's rank correlation test. For soil variables, clay % was correlated with sand % and silt %, and organic carbon % with bulk density (Annex 1-2). Clay %, as a measure of soil texture, organic carbon % (SOC), available water capacity (AWC), and depth of the bottom layer were retained. For landscape variables, SDI was found correlated with AWMSI, MPS, and NumP (Annex 3-4). SDI and MPAR were selected for further analysis.

Sites VT richness was estimated as the number of unique VT per site. The values ranged from 23 to 113 VT. To account for bias in sampling intensity between sites asymptotic taxa richness was estimated using the iNEXT package (Hsieh *et al.*, 2016). On average, extrapolated VT richness was 1.22 times greater than the observed one. Spearman's rank correlation test was used to explore the relation of the selected variables and the estimated VT richness.

Pairwise differences between AM fungal communities in each site were quantified by calculating Bray-Curtis dissimilarities using the package vegan (Oksanen *et al.*, 2020). Ward hierarchical clustering analysis was used to group communities based on the Bray-Curtis distance. Groups in the clustered communities were defined by inspecting at which number of

groups the correlation between the real distance matrix between sites and the distance matrix between the clusters maximizes, resulting in 5 groups of communities (Annex 5-6).

To compare the relative influences of our variables with fungal communities' similarity, and take into account geographical distance between sites, a general dissimilarity modeling (GDM) was used. GDM is a non-linear extension of matrix regression, designed to analyze and predict spatial patterns of turnover in community composition. The model uses splines to deal with two types of non-linearity found in ecological datasets: variation in the rate of community composition turnover across environmental gradients and the curvilinear relationship between community distance and environmental and geographical distance (Ferrier *et al.*, 2007). The GDM was created using the package gdm (Matthew Fitzpatrick *et al.*, 2021). Model and variable significance were tested using matrix permutations (50) and the default three I-splines. Model significance was determinate by comparing the deviance explained by GDM fitted with permuted and unpermuted matrices. To test the variable's significance this process was repeated for each variable individually. Variable importance was calculated as the percent change in deviance explain between a model fit with and without that variable permuted. The final model was selected using backward elimination, removing the variable with the lowest contribution value, and recalculating the model and variable significance as stayed before, until only significant variables were included (Ferrier *et al.*, 2007; Fitzpatrick & Keller, 2015; Matthew Fitzpatrick *et al.*, 2021).

Nonmetric multidimensional scaling (NMDS) was used to visualize the differences in the communities. Resultant significative variables from the GDM were fitted in the ordination.

4. RESULTS

4.1 AM fungal richness

A total of 24 sites with more than 20 records were analyzed. Estimated VT richness values ranged between 36 and 158 VT, with a mean value of 58. Estimated VT richness is visualized in Figure 3. The sites with higher estimated richness were located in western Estonia, with values in the range 36-140, including the site with the highest diversity, 140 VT. The estimated diversity of sites in south and eastern Estonia was in the range 26-56 VT, including the site with the lowest estimated richness. This indicates spatial variation in the diversity of AM fungal taxa across Estonia, with close sites having a similar richness.

Spearman correlation was used to test the correlation of the selected variables with the estimated VT richness. The test found significant correlations between the AM fungal VT richness and the depth of the soil layer (rho=-0.45, $p < 0.03$) and the landscape Shannon diversity (SDI) (rho=0.56, $p < 0.004$). This indicates a higher AM fungal richness in sites with a thinner top-soil layer, and sites with a contemporary diverse landscape. The other tested variables were not significantly correlated (Table 5), indicating that AM fungal taxa richness in the studied sites was not related to soil texture, measured with clay mass %, soil organic carbon content, water capacity, neither with the landscape change or landscape complexity, measured with MPAR.

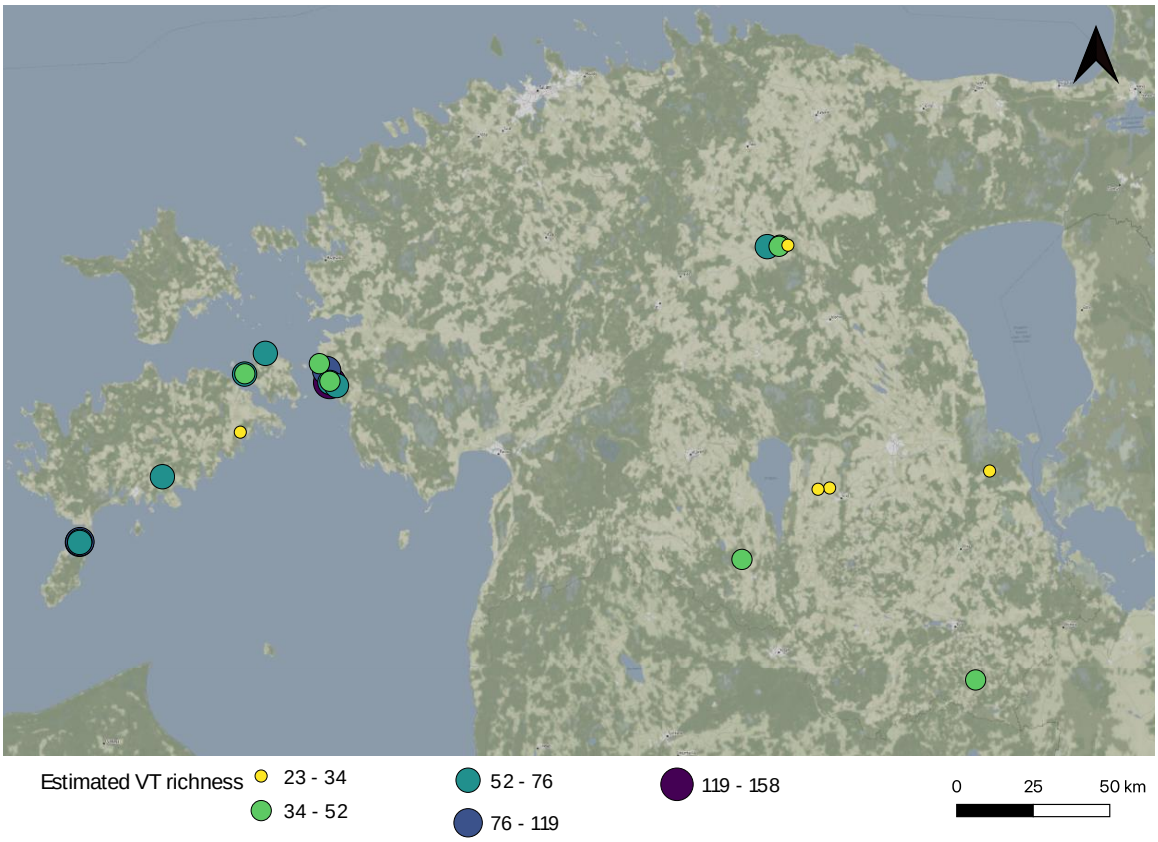

Figure 3. AM fungi estimated VT richness in each site. Symbol size and color indicate the estimated VT richness.

Table 5. Spearman correlation coefficient and p-value of estimated VT richness and selected soil and landscape structure and change in landscape variables.

Variable	Spearman coefficient	*p-value*
Clay %	-0.29	0.16
SOC %	0.09	0.67
AWC	-0.07	0.76

Depth of the soil layer (mm)	-0.45	**0.03**
SDI	0.56	**0.004**
MPAR	-0.16	0.46
Landscape change	0.13	0.53

Clay %: percentage mass of clay in fine-earth fraction, SOC %: soil organic carbon content percentage of soil weight, AWC: available water capacity mm H_2O/ mm soil, SDI: Shannon diversity index of the landscape; MPAR: Mean perimeter area ratio.

4.2 AM fungal community composition

AM fungal community composition similarity between sites was calculated using Bray-Curtis distance, and sites were clustered based on their community similarity. AM fungal community composition varied between sites as shown in Figure 4, displaying the richness and presence of VT that belong to a given genus differed between the resulted five cluster groups and within them.

The geographical distribution of the AM fungal community clusters is shown in Figure 5, where clusters one, three and four show spatial aggregation. This suggests that AM fungal communities from sites that are close in space are more similar than those that are more distant.

To test and model which of the selected soil, landscape, landscape change variables, and geographical distance were factors contributing to the community's dissimilarity, a GDM was used. Backward variable elimination in the GDM found three significant variables as predictors of the model: geographical distance (p<0.001) with a 24.04, soil clay % (p=0.04) with the importance of 18.60 and change in SDI (p=0.02) with importance of 10.86. This indicates that differences in the studied AM fungal communities are produced by the distance between sites, soil properties and change to a more diverse landscape. Depth of the soil layer, water capacity, soil organic carbon, and current landscape configuration were not significant factors to predict dissimilarity in the AM fungal community composition.

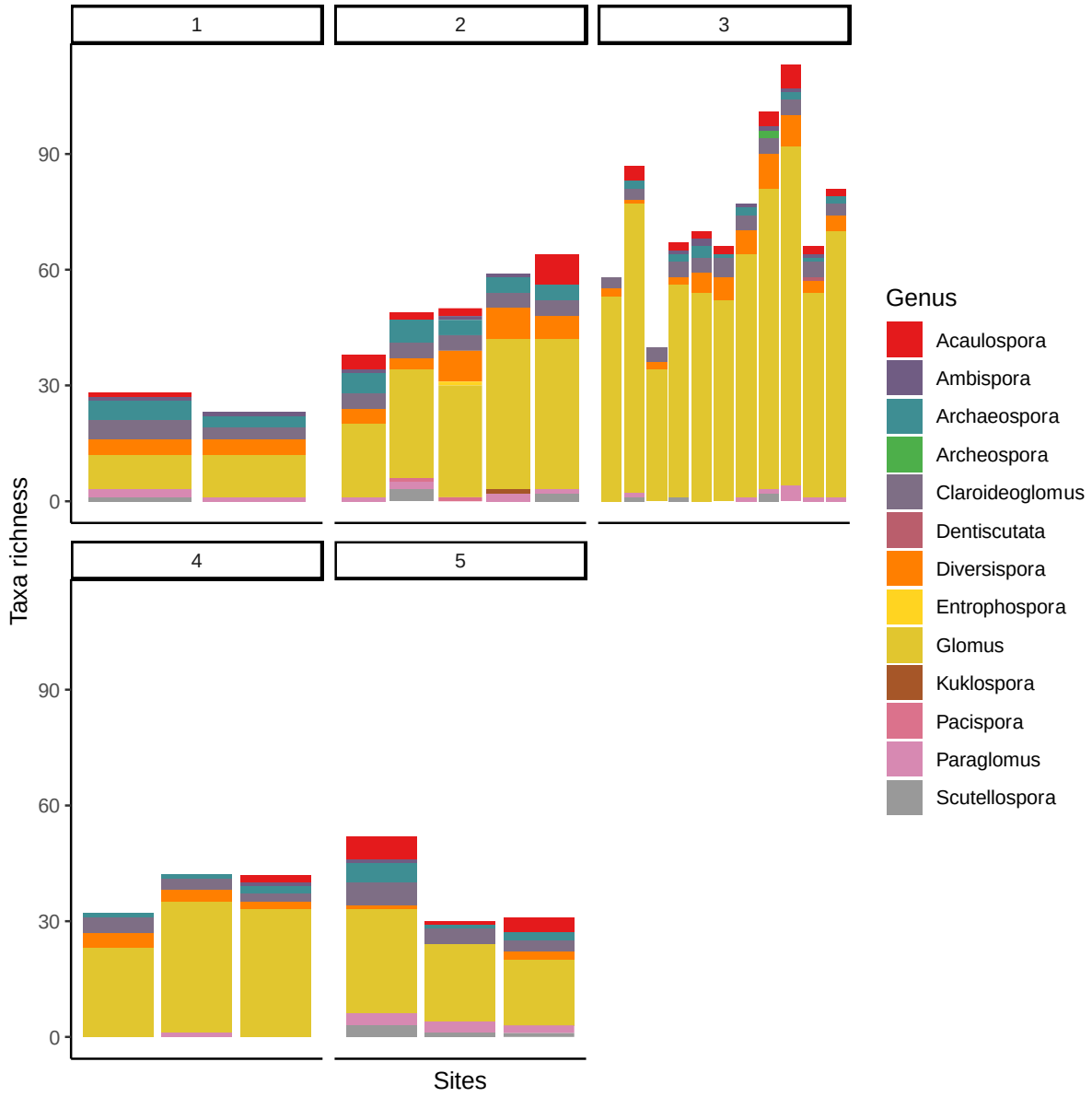

Figure 4. AM fungal taxa richness in each site and cluster group. Y-axis indicates the number of VT in each genus. Frames represent cluster groups based on the Bray-Curtis dissimilarity index.

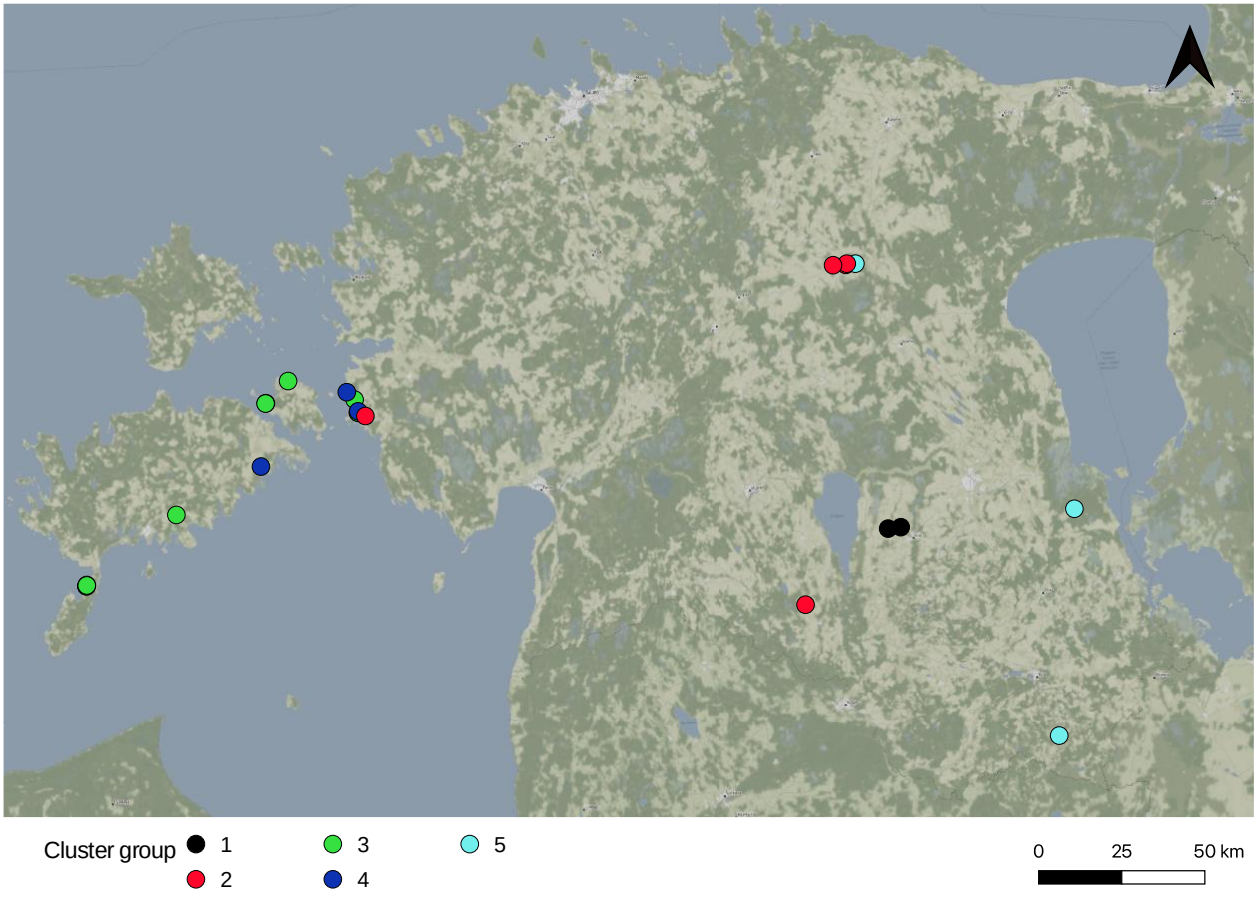

Figure 5. Distribution of AM fungal communities cluster groups. Color indicates belonging to a cluster group based on Bray-Curtis community similarity.

The GDM with the three significant predictors explained 46.37% of the deviance and was significant (p<0.001). Results of backward variable elimination and significance of the model and variables are provided in Annex 6-7. Splines of significative predictors were extracted, and values of the variables were scaled to be plotted together and allow comparison between them (Figure 6). For each spline, the slope represents the AM fungal community turnover along the variable gradient and the maximum height reached by each curve indicates the total amount of compositional turnover associated with that variable. As geographical distance between sites increases the AM fungal community dissimilarity also increases. Soils with small content of clay do not show big differences in AM fungal communities. It is in soils with high content of clay where differences produce a higher AM fungal community dissimilarity. Change of the landscape through time to be more diverse produced a small change in the AM fungal communities. The resulted model explained 46.37% of the deviance (Figure 7), suggesting that

other not tested variables, potentially not soil or landscape related, were responsible for the AM fungal community composition differences.

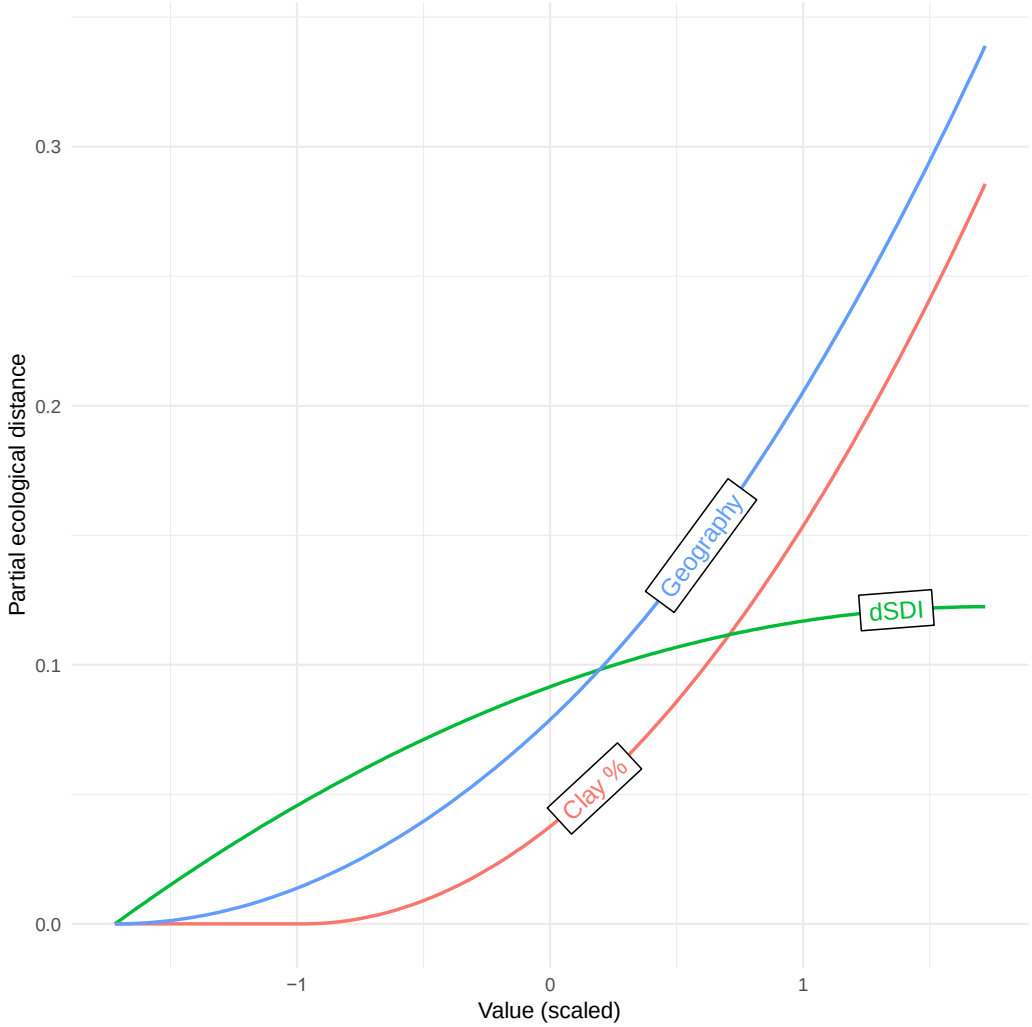

Figure 6. Results of GDM of AM fungal communities. Splines represent the community dissimilarly change associated with each significant variable over its range of values: Geography (geographical distance), Clay % (percentage mass of clay in fine-earth fraction) and dSDI (change in landscape SDI). Variables are scaled to compare spline shapes on the same x-axis.

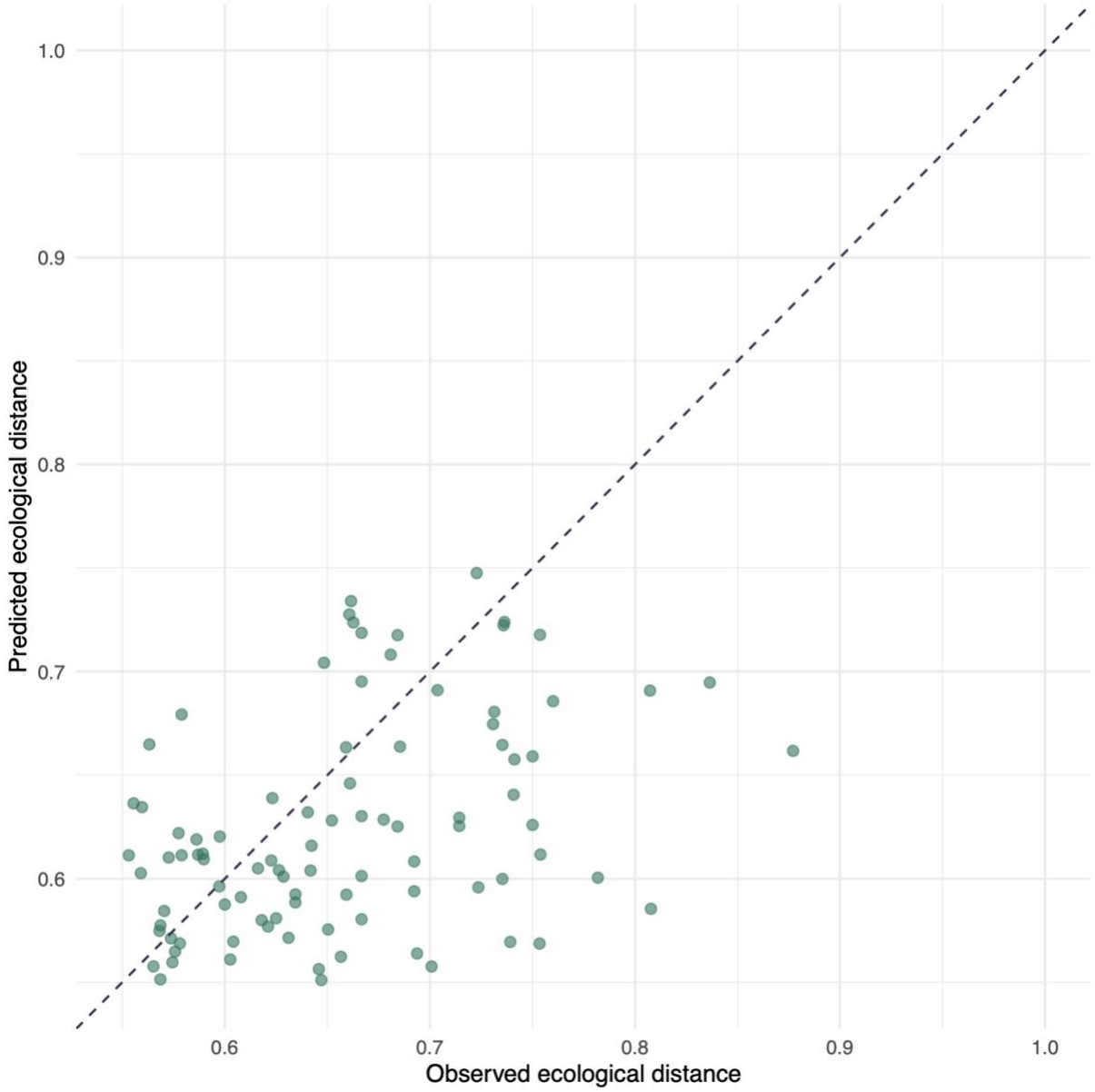

Figure 7. Observed ecological distances compared with values predicted by the GDM. The line represents perfect prediction.

NMDS was used to visualize the clustered AM fungal communities similarity in ordination space, and the resultant significant variables from the GDM were fitted in the ordination, alongside estimated AM fungal richness (Figure 8). AM fungal communities from sites located in western Estonia, with higher AM fungal VT richness, are plotted close just like in the real space (Figure 5). This correspondence to sites where the landscape has changed to be more diverse, and with soils with less clay content, being more sandy. On the other side, communities with low AM fungal diversity, found in eastern and central Estonia, are in soils with higher clay content and where the landscape has changed to be more dominant, with a lower SDI.

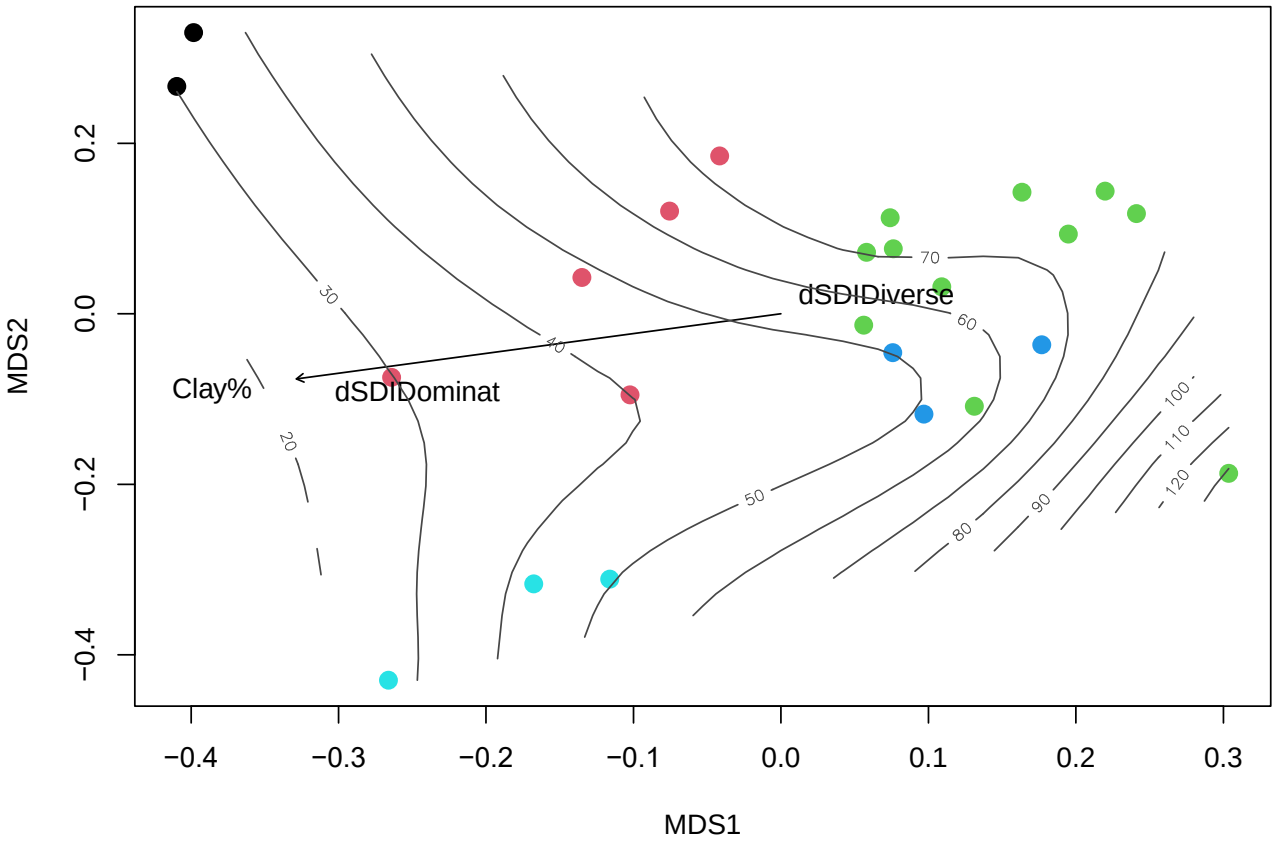

Figure 8. Non-metric multidimensional scaling (NMDS) ordination plot of AM fungal communities' dissimilarity. Color indicates belonging to a cluster group based on Bray-Curtis community similarity. Arrow shows direction of increasing soil clay content. dSDI values represent the centroids of the factor. Grey lines indicate the estimated Shannon diversity index.

5. Discussion

5.1 AM fungal diversity.

Abiotic and biotic filters determine the presence or absence of an AM fungal taxon in an ecosystem, resulting in differences in taxa richness and diversity, and community composition (Dumbrell *et al.*, 2010). In this study AM fungi VT richness and composition of 24 sites across Estonia were analyzed. Estimated VT richness range values, 26-140 VT, are higher than those reported by Sepp *et al.* (2018), 41-63 VT, who analyzed AM fungal diversity in 12 sites of Estonia, and are lower than the global range, 6-216 VT, reported by Pärtel *et al.* (2017). In terms of distribution, sites with the highest VT richness were located on western Estonia's coast, whereas sites with the lowest VT richness were located inland in eastern and south Estonia. Yet, variation in VT richness could be observed in spatially close sites. This, alongside with the low endemism patterns of AM fungal taxa (Davison *et al.*, 2015), and the capacity of this fungi to travel long by air (Chaudhary *et al.*, 2020) or biotic vectors (Correia *et al.*, 2019a) suggest that local variables were responsible for differences in taxa richness between sites.

The relation between AM fungal VT richness and soil and landscape configuration and change were tested. Soil texture, measured as clay percentage of the soil mass, soil organic carbon and water capacity were not significantly correlated with AM fungal VT richness. This contrast with earlier findings were soil texture (Klichowska *et al.*, 2019), and soil moisture (Chen *et al.*, 2017) serve as filter of AM fungal taxa, resulting in richness differences across contrasting soils. Depth of the top soil layer was significantly and negatively related with estimated AM fungal VT richness. Deeper soils can provide more water and nutrients to the plants, being an important factor of plant communities (Rajakaruna & Boyd, 2018). Distinct plant communities in terms of species and requirements host different AM fungi taxa (Xu *et al.*, 2016). The negative relation in AM fungal taxa richness could be the result of differences in plant communities species composition and traits growing in these soils rather than a direct effect of soil depth in AM fungi. Landscape diversity was positively and significantly correlated with VT richness. Higher values of this variable indicate a diverse landscape, with high number of small complex patches. For plants, landscape diversity is positively correlated with species diversity (Honnay *et al.*, 2003). Since AM fungal communities differ between plant communities (Klironomos, 2003), a diverse landscape with a high plant diversity could provide a wider range of hosts and habitats of AM fungi, resulting in a higher diversity. Finally, we found no correlation between the AM fungi richness and changes in the landscape. For fungi,

the effects of habitat fragmentation over time are poorly studied (Grilli *et al.*, 2017). For AM fungi changes in the landscape, more specifically loss our gain in forest area, have contrasting effects in AM fungi morphospecies richness (Mangan *et al.*, 2004; Grilli *et al.*, 2012). The results in this study indicate that AM fungal taxa richness in Estonia is driven by the current landscape configuration, the more diverse the landscape the more AM fungi taxa, and depth of the top soil layer, with deeper layers showing less AM fungal richness.

5.2 AM fungi community composition

Soil properties, climate, geographical distance, and plant community composition are some of the most important factors on the spatial structure of AM fungal communities (Jansa *et al.*, 2014; Davison *et al.*, 2015; Klichowska *et al.*, 2019). In this study we evaluated the importance of soil texture, landscape configuration, changes in landscape and geographical distance shaping the AM fungal community turnover using a GDM. Cluster groups of AM fungal communities visualized in the map indicated similarity of the communities of spatially close sites. In agreement with previous studies, a large part of the variation in AM fungal communities was given by the spatial separation between communities and soil properties (Jansa *et al.*, 2014; Klichowska *et al.*, 2019) and a small part of the variation was explained by changes in the landscape configuration. AM fungi have relatively efficient dispersal vectors (Correia et al., 2019; Chaudhary et al., 2020), and show low endemism (Davison *et al.*, 2015). The importance of geographical distance might be related with local biotic and abiotic factors variation, such as climate (Jansa *et al.*, 2014), or plant community composition (Xu *et al.*, 2016), rather than a dispersal limitation of AM fungi propagules.

AM fungal taxa have taxon specific responses to soil texture, resulting in communities differences even at local scale when the soil texture changes (Klichowska *et al.*, 2019). The results of this study found that soil texture, measured as the soil clay content, is a significant factor that explains differences in AM fungal communities of Estonia. Moreover, AM fungal communities of soils with high clay content differ more than communities of low clay content. Here again, besides the direct response of AM fungi to soil texture, observed differences could also be due to the effect of soil texture in plant communities species and traits. In relation with other tested soil properties, soil organic carbon, water availability and depth of the top soil layer were not significant predictors of the fungal communities turnover. Other studies have found these properties as drivers of AM fungi communities (Deepika & Kothamasi, 2014). Nevertheless, the importance of these variables is diminished when other variables are taken

into account (Klichowska *et al.*, 2019), showing its low prediction power in communities dissimilarity.

Habitat fragmentation affects the diversity and communities of organisms yet its importance in groups of fungi is not well studied (Grilli *et al.*, 2017). Here, we analysed the importance of the landscape configuration and changes in it, in AM fungi communities similarity. Although some AM fungal taxa are reported to respond differently to certain degrees of habitat fragmentation (Longo *et al.*, 2016). We found that neither current landscape diversity nor change in the landscape, measured as total change in landscape dissimilarity since the beginning of the 20th century, were significant predictors of the fungal communities dissimilarity. Nevertheless, change of the landscape to be more diverse or more dominant was a significant predictor. AM fungal communities of habitats under different land use intensities host different AM fungal taxa (Moora *et al.*, 2014), and communities are also the result of land use legacy (Faggioli *et al.*, 2019). Further analysis on how this change in landscape affects specific AM fungal taxa might help to understand the results of this study, providing information on how to maintain AM fungal taxa richness and which taxa that are resilient to change might be suitable to inoculate in fragmented landscapes.

Overall, we found that dissimilarity of AM fungal communities of Estonia is the result of spatial separation of the communities, differences in soil texture, and changes in the landscape configuration. Nevertheless, our model was only able to predict around 50 percent of the variability in the 24 studied communities. A systematic sampling across the country, that includes other well know variables that drive AM fungal community and more sampling sites composition should help to understand better the importance of the tested variables in the distribution of these fungi.

6. Conclusions

This study analysed the diversity and community composition of AM fungi Estonia and its relationship with soil and landscape properties. Taxa richness and community composition varied across sites in Estonia. We found that AM fungal taxa richness was related with the depth of the top-soil layer and the current landscape diversity. Differences in the community composition were explained by the distance between communities, soil texture and to lesser degree by changes in the landscape diversity.

Role of soil properties and landscape composition in arbuscular mycorrhizal fungi communities of Estonia

Oscar Zarate-Martinez

Summary

Arbuscular mycorrhizal (AM) fungi, subphylum Glomeromycotina (Spatafora *et al*., 2016), are a ubiquitous, abundant, and species-poor group of obligate symbionts of plant roots (Smith & Read, 2008). AM fungi gain all their carbon from their host plant while providing benefits for their host, such as improving soil nutrient acquisition and biotic and abiotic stress resistance (Smith & Read, 2008). The presence of the symbiosis can increase photosynthetic productivity and biomass accumulation in nutrient-deficient soils, making them an alternative for syntenic fertilizers (Begum *et al*., 2019). There are 342 (http://www.amf-phylogeny.com) morphological defined AM fungal species and between 500 to 1000 molecular defined taxa (Kivlin et al., 2011; Öpik et al., 2014). They have a cosmopolitan distribution, being found in most terrestrial ecosystems (Chaudhary et al., 2008), and have low endemism (Davison et al., 2015). However, AM fungal richness and community composition vary across ecosystems, with abiotic and biotic factors being responsible (Chaudhary et al., 2008). Abiotic factors, especially soil chemical (Dumbrell et al., 2010), physical (Klichowska et al., 2019), and nutrient availability (Camenzind et al., 2014), are crucial for AM fungal community composition. At large spatial scales, geographical distance between communities can describe the differences in AM fungal communities (Huang et al., 2019). Anthropogenic activities, especially those that modify ecosystems, are essential drivers of local biodiversity. For AM fungi, the type and intensity of land use affect their community composition (Moora et al., 2014). Still, the impact of modifications of the landscape configuration has been poorly studied (Bittebiere et al., 2020).

This thesis aimed to compare AM fungal communities' diversity and community composition in Estonia and analyze if soil physical properties, landscape configuration, and historical changes in the landscape could explain the differences in the AM fungal communities. AM fungal communities were described using records from the Maarj*AM* database (Öpik et al., 2010, https://maarjam.botany.ut.ee/). Records from ecological studies in Estonia were extracted, and communities were defined using the assigned Virtual Taxa (TV) in the database. A 1000 m buffer around each site with AM fungal records was created to analyze the importance of the landscape configuration. Topographic data from the Estonian Topographic

Database (ETAK) was used to model the features, land use types inside each landscape. Landscape metrics of diversity, shape complexity and edge density were calculated to test its relation in AM fungal richness and community composition. Similarly, to know the effect of changes in the landscape, historical maps from Estonia were revised. Features, land use types in the historical maps were digitalized for each site landscape in AM fungi records. Changes in the landscape diversity and dissimilarity of the landscapes across time were used to study their effect on AM communities. Soil properties for each site were extracted from the Est-Soil-EH v 1.0 database (Kmoch *et al.*, 2020). The correlation of AM fungal richness with the landscape, landscape change, and soil variables was tested. The importance and significance of these variables in communities turnover was assessed using a generalized dissimilarity model (GDM)

Spatial variation of AM fungi taxa richness was observed across 24 sites in Estonia. Sites with higher richness were observed in western Estonia, whereas lower richness was observed in south and eastern Estonia. Richness was significantly related to soil texture properties, depth of the topsoil layer and current landscape diversity, but not with changes in the landscape. GDM found three variables that could explain ca 50% of the variability in community composition: geographical distance between communities, soil clay content and change in the landscape diversity.

This study helped reinforce the importance of geographical distance and soil properties in AM fungal communities. Additionally, it provides evidence of the importance of the landscape configuration in AM fungal richness and the effect of historical changes in the landscape in AM fungal communities.

Mulla omaduste ja maastikustruktuuri mõju Eesti krohmseenekooslustele

Oscar Zarate-Martinez

Kokkuvõte

Krohm- ehk arbuskulaarmükoriissed (AM) seened (alamhõimkond *Glomeromycotina*; Spatafora jt, 2016) on laialtlevinud ja arvukas, kuid liigivaene rühm obligaatseid taimejuurte sümbionte (Smith ja Read, 2008). Krohmseened saavad kõik elutegevuseks vajaliku süsiniku taimedelt, pakkudes peremeestaimele vastutasuks efektiivsemat toitainete omastamist mullast ning vastupidavust biootilisele ja abiootilisele stressile (Smith ja Read, 2008). AM sümbioosi moodustamine võib suurendada taime fotosünteetilist aktiivsust ja biomassi toitevaestel muldadel, mistõttu võib AM olla alternatiiviks tehisväetistele (Begum jt, 2019). Morfoloogiliste tunnuste alusel on kirjeldatud 342 krohmseeneliiki ning molekulaarselt eristatakse 500 kuni 1000 taksonit (Kivlin jt, 2011; Öpik jt, 2014). Krohmseened on kosmopoliitse levikuga ning neid leidub enamikus maismaaökosüsteemides (Chaudhary jt, 2008), kusjuures endemismi esineb nende hulgas vähe (Davison jt, 2015). Siiski on leitud, et krohmseenekoosluste liigirikkus ja koosseis varieeruvad biootiliste ja abiootiliste tegurite mõjul ökosüsteemiti (Chaudhary jt, 2008). Abiootilistest faktoritest mõjutavad krohmseenekooslusi iseäranis mulla keemilised (Dumbrell jt, 2010), füüsikalised (Klichowska jt, 2019) ja toitainelised (Camenzind jt, 2014) omadused. Suuremas ruumimastaabis kirjeldab krohmseenekoosluste erinevusi geograafiline kaugus (Huang jt, 2019). Ökosüsteeme mõjutavast inimtegevusest on krohmseentele olulisimad faktorid maakasutuse tüüp ning intensiivsus (Moora jt, 2014), samas on maastikustruktuuri muutuste mõju küllaltki vähe uuritud (Bittebiere jt, 2020).

Käesoleva magistritöö käigus võrreldi krohmseenekoosluste liigirikkust ning koosseisu Eestis ning uuriti, kas kooslustevahelisi erinevusi on võimalik seletada mulla füüsikaliste omaduste, maastikustruktuuri või ajaloolise maakasutuse muutusega. Krohmseenekoosluste andmestik põhines andmebaasis Maarj*AM* (Öpik jt, 2010; https://maarjam.botany.ut.ee/) leiduvatel Eestis tehtud ökoloogiliste uuringute kirjetel, kus kooslused on kirjeldatud sealesinevate krohmseente virtuaaltaksonite (VT) kaudu. Maastikustruktuuri mõju uurimiseks kasutati 1000 m puhvrit ümber iga prooviala, mille piires kirjeldati Eesti topograafia andmekogu (ETAK) põhjal maastikuelemendid ning maakasutuse tüübid ning arvutati maastikulise mitmekesisuse, keerukuse ja servatiheduse indeksid. Samuti kasutati maastikumuututse mõju uurimiseks ajaloolisi kaarte, kusjuures iga prooviala piires digiteeriti maastikuelemendid ja maakasutuse

tüübid. Mullaparameetrite kirjed saadi andmebaasist Est-Soil-EH v1.0 (Kmoch jt, 2020). Uuriti eelmainitud keskkonnaparameetrite mõju krohmseente liigirikkuse ja koosluse koosseisu kujundamisel, mõju mastaabi hindamiseks kasutati üldistatud erinevusanalüüsi (*generalized dissimilarity model*, *GDM*). Kromseente liigirikkus erines 24 prooviala lõikes geograafiliselt – Lääne-Eesti proovialad olid liigirikkamad kui Lõuna- ja Ida-Eesti proovialad. Liigirikkus oli statistiliselt oluliselt seotud mullastruktuuri, orgaanilise horisondi tüseduse ning kaasaegse maastikulise mitmekesisusega, kuid ei sõltunud maastikumuutuste ajaloost. Krohmseenekoosluste koosseisu mõjutajatena tuvastas *GDM* kolm tegurit, mis kirjeldasid *ca* 50% muutustest: kooslustevaheline geograafiline kaugus, savi sisaldus mullas ning muutus maastikulises mitmekesisuses.

Magistritöö kinnitas geograafilise kauguse ning mullaparameetrite olulisust krohmseenekoosluste kujundamisel. Lisaks nähtub tööst maastikustruktuuri mõju krohmseente liigirikkusele ning ajalooliste maastikumuutuste mõju krohmseenekoosluste koosseisule.

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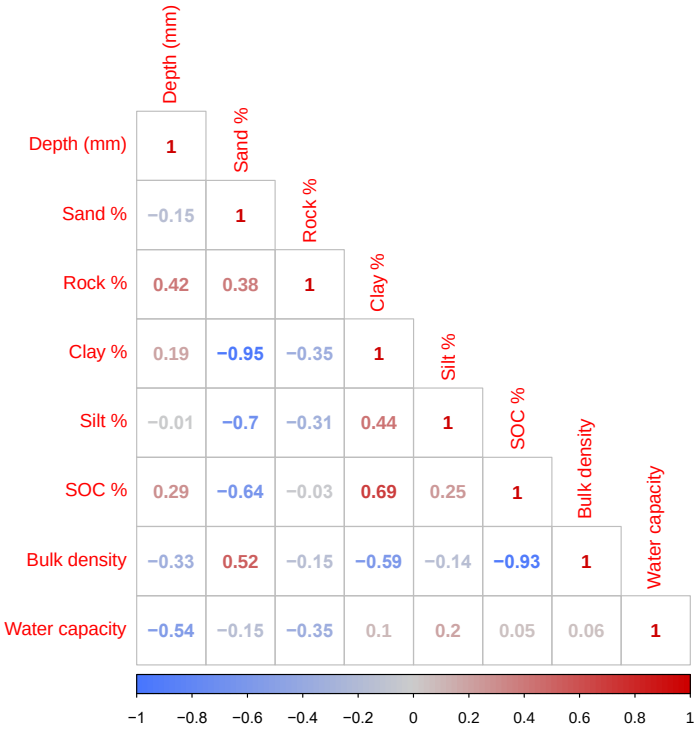
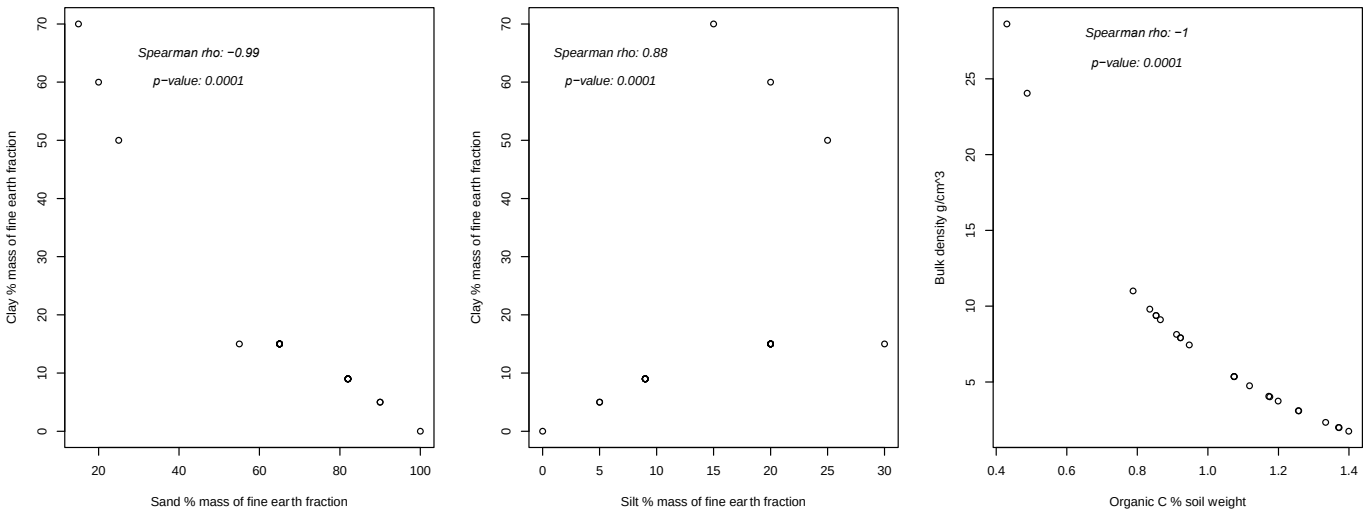
8. ANNEXES

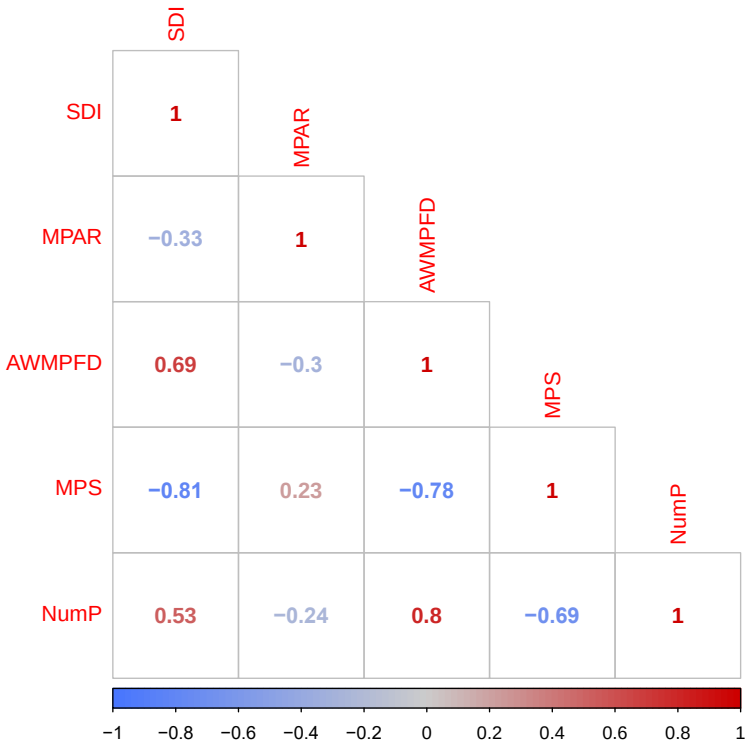
Annex 1. Correlations of all soil variables.



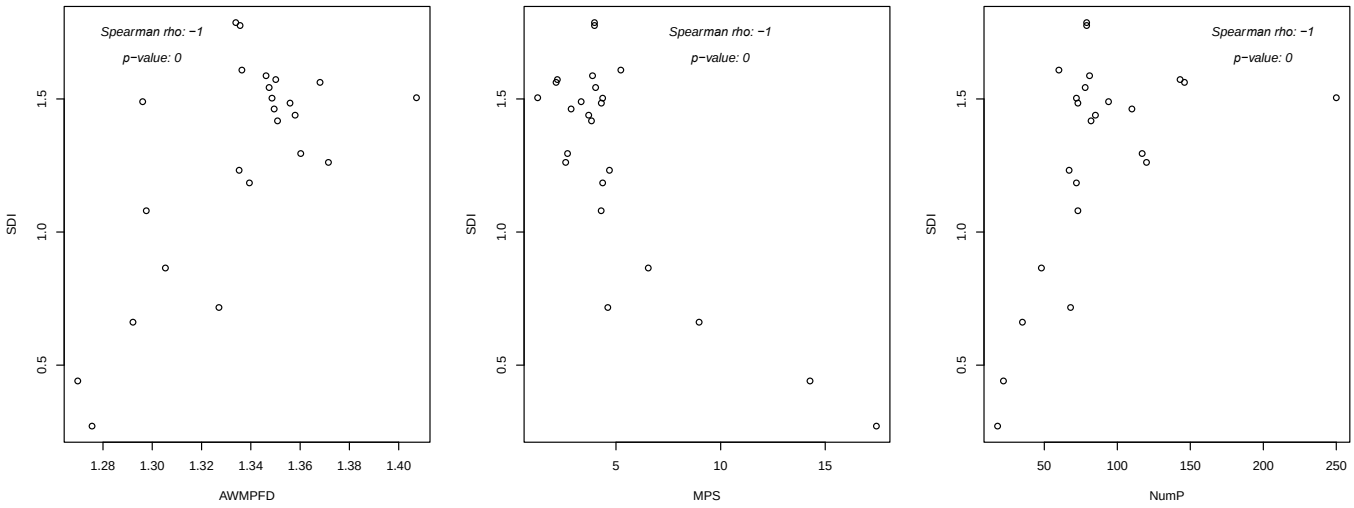
Annex 2. Significative correlations of soil variables.



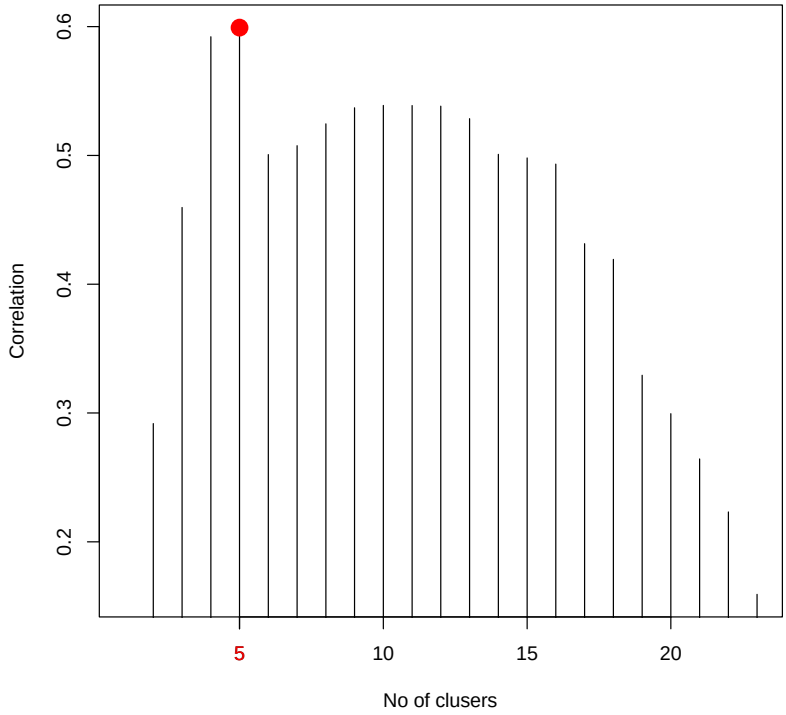
Annex 3. Correlations of all landscape metrics.



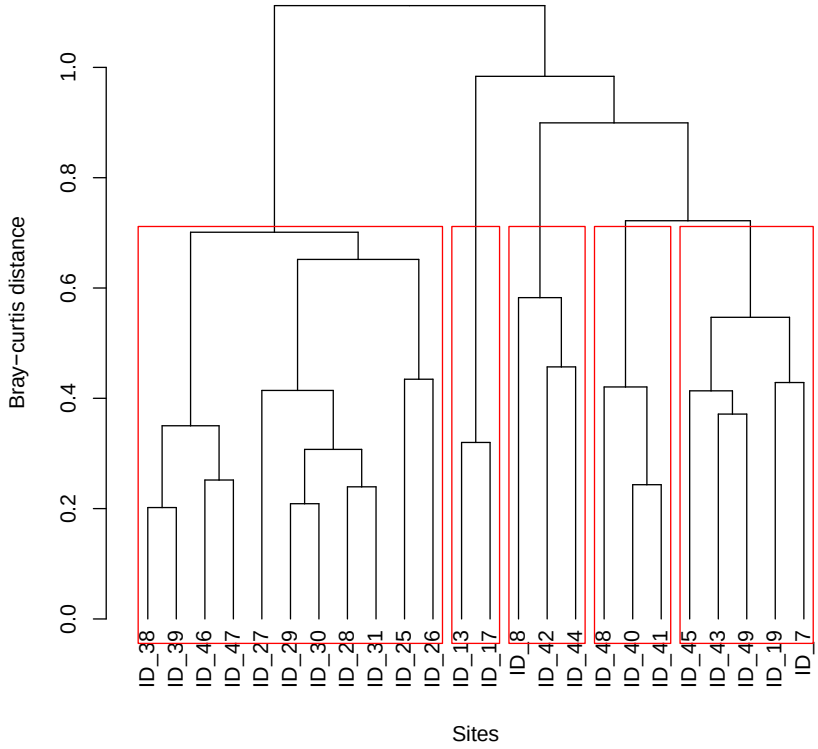
Annex 4. Significative correlations of landscape metrics.



Annex 4. Correlation between the real distance matrix between sites and the distance matrix while iterating from 2 to the total number of sites (24). The red dot indicates the maximum correlation value.



Annex 5. Dendrogram for produced clusters using Bray-Curtis distance. Red squares indicate cut when assigning 5 groups.


Bray−curtis distance
0.0
0.2
0.4
0.6
0.8
1.0
ID_38
ID_39
ID_46
ID_47
ID_27
ID_29
ID_30
ID_28
ID_31
ID_25
ID_26
ID_13
ID_17
ID_8
ID_42
ID_44
ID_48
ID_40
ID_41
ID_45
ID_43
ID_49
ID_19
ID_7
Sites

Annex 6. GDM significance through the backward elimination process.

	Model	Model - MPAR	Model – MPAR - depth soil	Model – MPAR – depth soil-landscape change	Model – MPAR – depth soil-landscape change SOC	Model – MPAR – depth soil-landscape change SOC-AWC
Model deviance	9.09	9.08	9.41	9.78	10.39	11.03
Percent of deviance explained	49.75	49.75	49.75	47.92	45.93	42.52
p-value	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005

Clay %: percentage mass of clay in fine-earth fraction, SOC %: soil organic carbon content percentage of soil weight, AWC: available water capacity mm H_2O/ mm soil, SDI: Shannon diversity index of the landscape; MPAR: Mean perimeter area ratio.

Annex 7. Variables importance and significance in GDM through the backward elimination process.

	Full Model		´ Model 1		Model 2	
Variable	Variable importance	Variable significance	Variable importance	Variable significance	Variable importance	Variable significance
Geography	12.72	< 0.05	12.72	< 0.05	14.78	< 0.05
Clay %	9.41	0.12	9.41	0.06	9.41	0.12
SOC %	4.46	0.18	4.46	0.26	4.46	0.14
AWC	2.89	0.12	2.89	0.26	3.38	0.24
Depth soil	0.00	0.66	0.00	0.76	-	-
SDI	11.52	0.12	11.52	0.08	11.51	0.10
MPAR	0.00	1.00	-	-	-	-
Change in landscape distance	3.69	0.16	3.69	0.20	3.69	0.26
Change in SDI	4.23	0.08	4.23	0.06	4.23	0.04

	Model 3		´ Model 4		Model 5	
Variable	Variable importance	Variable significance	Variable importance	Variable significance	Variable importance	Variable significance
Geography	16.07	< 0.05	22.72	< 0.05	25.22	< 0.05
Clay %	12.01	0.06	13.04	0.04	12.24	0.12
SOC %	4.15	0.20	NA	-	-	-
AWC	6.21	0.04	7.41	0.18	-	-
Depth soil	-	-	-	-	-	-
SDI	10.12	0.08	9.28	0.04	8.30	0.24
MPAR	-	-	-	-	-	NA
Change in landscape distance	-	-	-	-	-	-
Change in SDI	4.49	0.04	6.58	< 0.05	6.94	0.04

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