Improved description of terrestrial habitat types by including microbial communities as indicators

Danielsen, Anne-Cathrine Storgaard; Nielsen, Per Halkjær; Hermansen, Cecilie; de Jonge, Lis Wollesen; Jørgensen, Vibeke Rudkjøbing; Greve, Mogens Humlekrog; Corcoran, Derek; Dueholm, Morten Kam Dahl; Bruhn, Dan; Weber, Peter Lystbæk

Published in:
Journal of Environmental Management

DOI (link to publication from Publisher):
10.1016/j.jenvman.2023.118677

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

Journal of Environmental Management 344 (2023) 118677

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Improved description of terrestrial habitat types by including microbial communities as indicators

Anne-Cathrine Storgaard Danielsen [a,b,*], Per Halkjær Nielsen [c], Cecilie Hermansen [a,b], Peter Lystbæk Weber [a], Lis Wollesen de Jonge [a,b], Vibeke Rudkjøbing Jørgensen [c], Mogens Humlekrog Greve [a,b], Derek Corcoran [b], Morten Kam Dahl Dueholm [c], Dan Bruhn [d]

[a] Section of Soil Physics and Hydropedology, Department of Agroecology, Aarhus University, Blichers Allé 20, 8830, Tjele, Denmark
[b] SustainScapes - Center for Sustainable Landscapes Under Global Change, Department of Biology, Aarhus University, Nordre Ringgade 1, 8000, Aarhus C, Denmark
[c] Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Frederik Bajers Vej 7H, Aalborg, DK 9220, Denmark
[d] Section of Biology and Environmental Science, Department of Chemistry and Bioscience, Aalborg University, Frederik Bajers Vej 7H, Aalborg, DK 9220, Denmark

ARTICLE INFO

Handling Editor: Lixiao Zhang

Keywords:
Macroflora
Soil properties
Ordination analysis
Grassland
Heathland
Environmental monitoring

ABSTRACT

Soils host diverse communities of microorganisms essential for ecosystem functions and soil health. Despite their importance, microorganisms are not covered by legislation protecting biodiversity or habitats, such as the Habitats Directive. Advances in molecular methods have caused breakthroughs in microbial community analysis, and recent studies have shown that parts of the communities are habitat-specific. If distinct microbial communities are present in the habitat types defined in the Habitats Directive, the Directive may be improved by including these communities. Thus, monitoring and reporting of biodiversity and conservation status of habitat types could be based not only on plant communities but also on microbial communities. In the present study, bacterial and plant communities were examined in six habitat types defined in the Habitats Directive by conducting botanical surveys and collecting soil samples for amplicon sequencing across 19 sites in Denmark. Furthermore, selected physico-chemical properties expected to differ between habitat types and explain variations in community composition of bacteria and vegetation were analysed (pH, electrical conductivity (EC), soil texture, soil water repellency, soil organic carbon content (OC), inorganic nitrogen, and in-situ water content (SWC)). Despite some variations within the same habitat type and overlaps between habitat types, habitat-specific communities were observed for both bacterial and plant communities, but no correlation was observed between the alpha diversity of vegetation and bacteria. PERMANOVA analysis was used to evaluate the variables best able to explain variation in the community composition of vegetation and bacteria. Habitat type alone could explain 46% and 47% of the variation in bacterial and plant communities, respectively. Excluding habitat type as a variable, the best model (pH, SWC, OC, fine silt, and Shannon's diversity index for vegetation) could explain 37% of the variation for bacteria. For vegetation, the best model (pH, EC, ammonium content and Shannon's diversity index for bacteria) could explain 25% of the variation. Based on these results, bacterial communities could be included in the Habitats Directive to improve the monitoring, as microorganisms are more sensitive to changes in the environment compared to vegetation, which the current monitoring is based on.

1. Introduction

Biodiversity is in crisis on a global scale (Ejrnæs, 2011; Thaler, 2021). Up to one million animal and plant species are at risk of extinction within the coming decades. This, along with the loss of natural habitats, poses a threat to ecosystem services such as pollination, food production, and air and climate regulation (Bongaarts, 2019). In contrast to 'macro-organisms', such as plants and animals, little is known about the trends in microbial biodiversity (Cavicchioli et al., 2019; Thaler, 2021); nor is it clear whether the trend is an increase or a decrease in diversity over time (Thaler, 2021). This knowledge gap contrasts with the fact that terrestrial microorganisms form the cornerstone of several ecosystem functions at the global level (Chu et al., 2020; George et al., 2019; Jansson and Hofmockel, 2020) and are

* Corresponding author.Section of Soil Physics and Hydropedology, Department of Agroecology, Aarhus University, Blichers Allé 20, 8830, Tjele, Denmark.
E-mail address: acsd@agro.au.dk (A.-C.S. Danielsen).

https://doi.org/10.1016/j.jenvman.2023.118677
Received 24 May 2023; Received in revised form 15 July 2023; Accepted 25 July 2023
Available online 7 August 2023

fundamental in maintaining a healthy soil. Furthermore, predicting the potential consequences of a changing climate is difficult due to the lack of baseline knowledge regarding the microbial communities (Kuramae et al., 2012).

Soils host a vast diversity of microorganisms, but only a small fraction of these microorganisms has been described (Dance, 2020; Delgado-Baquerizo et al., 2018). Furthermore, microorganisms are rarely included in nature protection and management policies (Cavicchioli et al., 2019). This is despite the fact, that belowground diversity is crucial for the functioning of aboveground diversity (Bardgett & Van Der Putten, 2014) and, therefore, highly relevant to include.

In the European Union, the Member States are obliged, through the Habitats Directive, to protect and preserve selected species and habitats. This must be done through the designation of protected habitats and continuous monitoring and regular reporting of the state of nature. The habitat types are listed in the Habitats Directive Annex I and differ with respect to soil physico-chemical properties, floristic composition, and diversity.

The current management and monitoring of the habitat types rely heavily on botanical surveys as well as some soil properties, for example, pH, soil water content, and carbon/nitrogen-ratio. A set of characteristic plant species has been defined for each habitat type and the presence of these is used to identify the habitat types. However, characteristic species are not always present, and the boundaries between habitat types are not always clear-cut, as some habitat types form mosaics with similar types (Commission, 2013). This complicates the identification of habitat types and the distinction between different types. Microbial communities could be used as a supplement to the present monitoring and assessment if distinct microbial communities are present in the habitat types. This could assist in the monitoring of habitat types, possibly resulting in a faster and more precise identification. Furthermore, microbial communities respond quickly to changes in the surrounding environment, compared to vegetation (Fierer et al., 2021; Lauber et al., 2013). Including the microbial community and its relation to a range of soil physico-chemical properties would therefore make it possible to detect environmental changes faster and implement activities to mitigate possible negative effects. The DNA present in the soil could be used to characterize microbial communities (Ladin et al., 2021), which would make it possible to include microorganisms in the current fieldwork related to the monitoring and assessment of the European habitat types as well as in similar monitoring programs. While many species of plants and animals as used as indicators to detect environmental change (bioindicators) (e.g. Theron et al., 2022; Areco et al., 2021; Grau-Andrés et al., 2019; Sanchez-Hernandez, 2006), microbial indicators are less frequently used (Ma et al., 2022).

In the present study, bacteria present in the soil, a part of the soil microbiome, and vegetation were examined in six habitat types from the Habitats Directive. Only few studies have examined the bacterial communities in these habitat types and, to the knowledge of the authors, not with the purpose of using the communities as bioindicators. Plant and bacterial communities were examined by analysing the alpha diversity and dissimilarity in community composition (beta diversity) between habitat types. Alpha diversity describes the diversity at a local scale (Andermann et al., 2022; Whittaker, 1960) while beta diversity describes the dissimilarity between species composition at different sites (Andermann et al., 2022). Furthermore, selected soil physico-chemical properties were examined. Properties expected to influence the variation in the biological communities were selected with the purpose of examining whether they varied between habitat types and which properties were most important in shaping the biological communities. The selected soil physico-chemical properties correspond to those included in the national monitoring of the habitat types, but additional soil properties were analysed, including soil texture, organic carbon content (OC), the degree of potential water repellency (WR), in-situ water content (SWC), and electrical conductivity (EC). Hydrophilic (readily wettable) soils are characterized by a surface tension higher than 71.27 mN m^{-1}, while hydrophobic (water repellent) soils exhibit surface tensions less than 71.27 mN m^{-1} (Roy and McGill, 2002). A link has recently been found between WR, soil properties and the diversity of plants and microorganisms (Seaton et al., 2019). To the knowledge of the authors, only very few studies have examined the variation in WR within the European habitat types, but it has been observed in grasslands and heathlands in previous studies (e.g., Sándor et al., 2021; Seaton et al., 2019; Martínez-Zavala and Jordán-López, 2009).

The fact that soil physico-chemical properties and the diversity and type of vegetation differ between the habitat types forms the basis for the central hypothesis in this study; that the different habitat types accommodate characteristic bacterial communities. To test this hypothesis, thorough examinations of soil properties and plant and bacterial communities were conducted in six habitat types with the aim of investigating the following objectives:

(i) Whether the selected habitat types differ with respect to specific soil physico-chemical properties as expected
(ii) If there is a correlation between the diversity of vegetation and bacteria present in the soil within selected habitat types
(iii) Whether bacteria present in the soil show specificity to one of the six habitat types
(iv) Which combination of variables, including habitat type, soil physico-chemical properties, and diversity indices, could best explain the community composition of plants and bacteria, respectively

The first objective addresses the representativeness of the selected habitat types and whether the soil properties differ between habitat types, as expected. Some of the habitat types contain naturally species-rich plant communities, while others support a lower plant species richness. This makes it possible to examine whether habitat types with a high plant species-richness also contain a high bacterial richness and vice-versa, which is addressed in the second objective. The third objective is concerned with the habitat specificity of the plant and bacterial communities. It is examined whether the habitat types are reflected in the community composition of plants, as expected, and bacteria. Lastly, the analysis of selected soil physico-chemical properties makes it possible to investigate the relation between the inherent soil properties and the biological communities, addressed in the fourth objective.

2. Materials and methods

To examine if habitat-specific communities exist in the habitat types, botanical surveys and amplicon sequencing were used to examine plant and bacterial communities, respectively. This data was used to calculate alpha and beta diversity. Alpha diversity data was used to examine objective (ii) and beta diversity to examine objective (iii) and (iv). To verify that the soil properties differed between habitats as expected based on the descriptions of the habitat types (objective i), soil samples were collected, analysed and compared to results from the national monitoring in Denmark. Furthermore, the results were also used to examine objective (iv).

2.1. Habitat types and field sampling design

Six different habitat types from the European Habitats Directive were examined, comprising three types of grasslands and three types of heathlands (Table 1). In the present study, the term habitat type is used as an environmental classification and refers to a specific assemblage of abiotic characteristics and the associated plant communities. A description of the habitat types can be found in the Interpretation Manual of European Union Habitats (Commission, 2013).

A total of 19 different locations (three to four locations representing each habitat type) were examined (Fig. 1A). Within each location, field

Table 1
Overview of the habitat types examined. The official name of each habitat type can be found in the first column. In the second column, the abbreviated names used throughout the present study are given and the NATURA 2000-code for each habitat type is listed in the third column. Natura 2000-codes and official names were found in Natura (2016) and European Commission (2013).

Official name	Abbreviated name	NATURA 2000-code[a]
Species-rich Nardus grasslands, on silicious substrates in mountain areas (and submountain areas in Continental Europe)	Calcareous grassland	6210
Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*)	Acidic grassland	6230
Xeric sand calcareous grasslands	Sandy grassland	6120
European dry heaths	Dry heath	4030
Northern Atlantic wet heaths with *Erica tetralix*	Wet heath	4010
Decalcified fixed dunes with *Empetrum nigrum*	Fixed dune	2140

[a] NATURA 2000-codes from Annex I of the Habitats Directive.

surveys were conducted at three different sites (the circles on Fig. 1B) to cover the variation within each location. Each site consisted of a circle (Fig. 1C) with a radius of 5 m (= 78.5 m^2). A minimum distance of 20 m was kept between sites (Fig. 1B). Locations were selected based on data from the ongoing mapping and monitoring of terrestrial habitat types in Denmark (Nygaard et al., 2016), and the geographical locations were found on the Danish Environmental Portal's website (Naturdata, 2020). In the field, the previous habitat determination was confirmed or denied using descriptions of the habitats (Buchwald, 2000; Natura, 2016) and the Habitat Key (Habitatnøgle, 2016). Only locations identifiable as one of the six habitat types were included. In five locations, it was only possible to examine two sites due to the limited area of the habitat type in question. All field surveys were conducted in August and September 2020.

2.2. Botanical surveys

Within each site (Fig. 1C), botanical surveys were conducted to examine species richness and frequency of the individual plant species. Species richness was determined by identifying all vascular plant species within each site to species level using selected literature (Faurholdt and Schou, 2012; Mossberg and Stenberg, 2014; Schou, 2006; Schou et al., 2010, 2014; Seberg et al., 2012). At three different places within each site, a pinpoint frame was positioned and used to determine plant species frequency (Levy and Madden, 1933). The pinpoint frames were positioned so they covered the highest diversity as well as the variation within each site.

2.3. Soil sampling and analysis of soil properties

Soil samples were collected at 0–16 cm depth at the centre of each pinpoint frame (resulting in three samples per site), after removal of vegetation and litter, and kept in sealed bags in a cooler box. In the laboratory, the three soil samples per site were pooled, resulting in a total of 52 soil samples (one sample per location) and these samples were each divided into two subsamples: one for amplicon sequencing and one for analysis of soil properties. The subsamples for sequencing were kept at −20 °C until analysis.

Samples for analysis of soil properties were kept at 5 °C until the following day where the in-situ soil-water content (SWC) and inorganic nitrogen content (NH_4^+ and $NO_3^- + NO_2^-$) were measured. The SWC was measured after oven-drying at 105 °C for 24 h (Danish Standard, DS/EN ISO 17892). To analyse inorganic nitrogen content, 10 g soil was mixed with 50 mL 1 M potassium chloride, the solution was centrifuged (ScanSpeed, 1236, 2000 rpm for 5 min), filtered (fibreglass filter, pore size 0.7 μm), and finally analysed on an autoanalyzer (Technicon, TRAACS 800). The remaining soil was dried and sieved to 2 mm prior to analysis of pH (in a soil/water suspension of 1:5 (v/v)), electrical conductivity (EC) (determined in a soil/water suspension of 1:10 (v/v)), and potential soil water repellency (WR). Before WR was measured, the samples were oven-dried at 60 °C and equilibrated for 48 h at 20 °C as described in Hermansen et al. (2019). The degree of WR was measured using the MED test (de Jonge et al., 1999, 2007; King, 1981). Soil OC was measured with an ELTRA Helios C-analyzer (ELTRA GmbH) after removal of carbonates by hydrochloric acid. Soil texture was measured by wet sieving and the pipette method (Gee and Or, 2002) and the particle size fractions were classified according to the International Soil Science Society classification system (clay (<0.002 mm), silt (0.002–0.02 mm) and sand (0.02–2 mm)).

2.4. DNA extraction

DNA was extracted using the DNeasy PowerSoil Pro QIAcube HT Kit, following the manufacturer's instructions (Qiagen, 2019), purified using a QIAcube Connect in an automated setup and finally eluted in 120 μL buffer. DNA-concentrations were measured using the Qubit dsDNA HS kit (Invitrogen) and a Tecan Infinite M1000 PRO plate reader (at 485/530 nm).

2.5. 16 S rRNA gene amplicon sequencing

A two-step PCR cycle was used to first amplify the V4 variable region of the 16 S rRNA genes and secondly to prepare the amplicons for sequencing by adding adapters and barcodes. The first reaction used the 515 F (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG- GTGYCAGCMGCCGCGGTAA-3′) (Parada et al., 2016) and 806 R (5′-TCGTCGGCAGCGTC AGATGTGTATAAGAGACAG-GGACTACNVGGGTWTCTAAT-3′) (Apprill et al., 2015) primers with overhangs for the subsequent barcoding. 25 μL PCR reactions in duplicate were run for each sample using 1X PCRBIO Ultra Mix (PCR Biosystems), 400 nM of both forward and reverse primer, and 10 ng template DNA. Samples were diluted with nuclease-free water if the concentration exceeded 5 ng/μL to avoid PCR inhibition (Karst et al., 2016). PCR conditions were 95 °C, for 2 min followed by 30 cycles of 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 50 s, followed by a final elongation at 72 °C for 5 min. PCR products were purified using 0.8x AMPure XP beads and eluted in 25 μL nuclease-free water. 2 μL of purified PCR product from above was used as template for a 25 μL Illumina barcoding PCR reaction containing 1x PCRBIO Reaction buffer, 1 U PCRBIO HiFi Polymerase (PCR Biosystems) and 10 μL of Nextera adaptor mix (Illumina). PCR conditions were 95 °C, for 2 min, 8 cycles of 95 °C for 20 s, 55 °C for 30 s, and 72 °C for 60 s, followed by a final elongation at 72 °C for 5 min. PCR products were purified using 0.8x AMPure XP beads and eluted in 25 μL nuclease-free water. DNA concentrations were determined as described for the extracted DNA.

The amplicons were pooled in equimolar concentration, and pair-end sequenced using an Illumina MiSeq (Illumina, USA) and a MiSeq Reagent kit v3 (Illumina, USA) aiming at 60,000 reads per sample.

Usearch v.11.0.667 (Edgar, 2010) was used to process the amplicon data. 16 S rRNA gene V4 forward and reverse reads were filtered to remove phiX sequences using usearch -filter_phix, merged using the usearch -fastq_mergepairs command, and quality filtered using usearch -fastq_filter with -fastq_minlen 200 and -fastq_maxee 1.0. Dereplication was performed using -fastx_uniques with -sizeout, unique read with <60% identity to reference reads in the SILVA 138.1 SSURef NR99 database (Quast et al., 2012) were removed using -usearch_global with -strand both, -id 0.6, -maxaccepts 1, -maxrejects 8, and -matched. Amplicon sequence variants (ASVs) were resolved using the usearch -unoise3 command. An ASV-table was created by mapping the quality filtered reads to the ASVs using the usearch -otutab command with the -zotus and -strand plus options. Taxonomy was assigned to ASVs based on the SILVA 138.1 SSURef NR99 database using the usearch -sintax command with -strand both and -sintax_cutoff 0.8 options. An overview

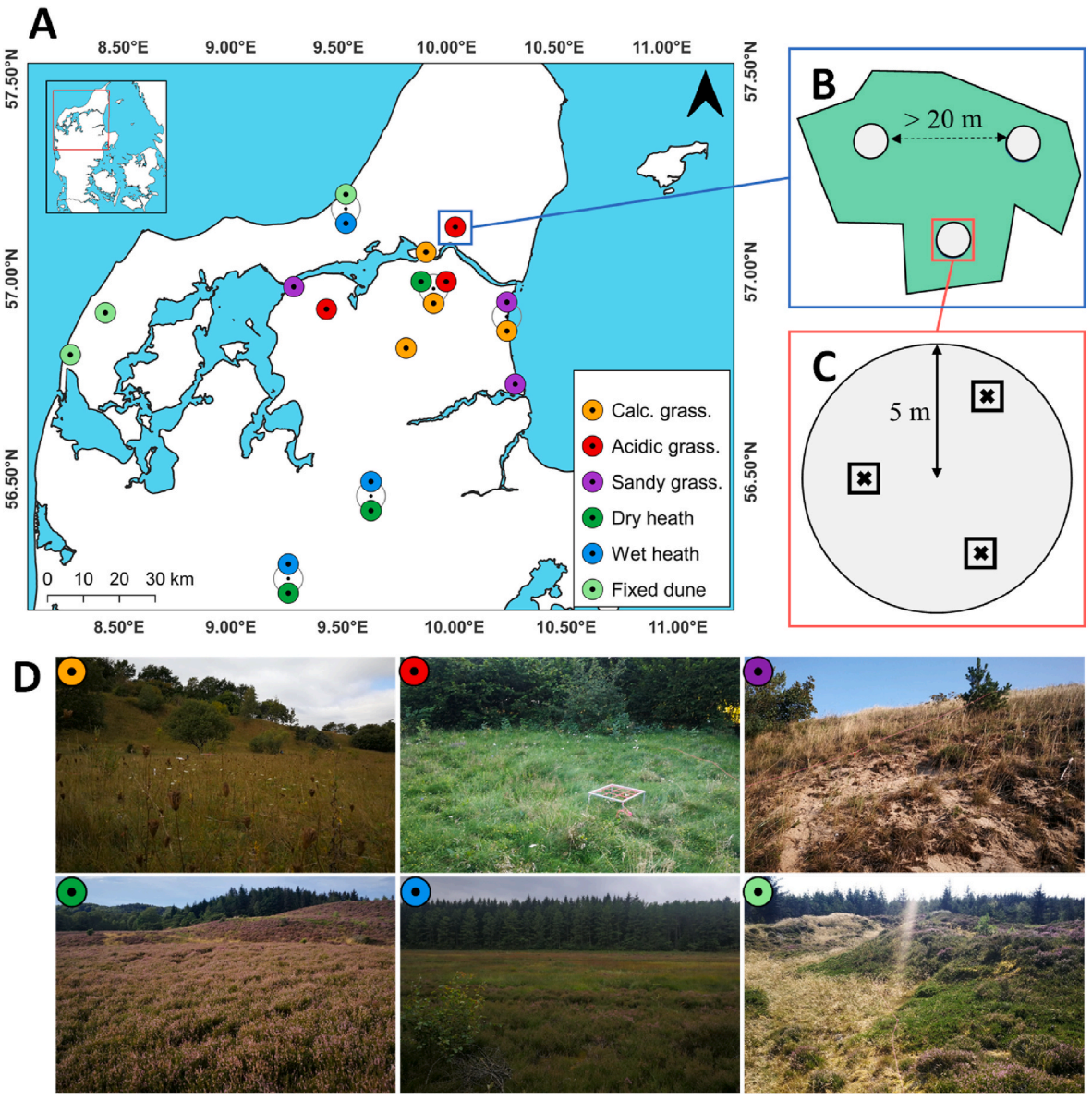

Fig. 1. The geographical position of study areas, i.e. locations (A), illustration of the sampling design within each location, each circle corresponds to a site (B), illustration of one site (C), and images of the habitat types (D) taken by the first author during fieldwork. Each coloured circle on the map (A) corresponds to one location. Five of the locations were located too close to show on the map; a colourless circle connects these locations, and the connected locations were in the centre of the colourless circle. The polygon seen in (B) illustrates a location and corresponds to one of the coloured circles in (A). The three circles within the location seen in (B) illustrate the three sites where field surveys were conducted. One of these sites is illustrated in (C), where the squares indicate pinpoint frames and x marks where soil samples were collected.

of the sequencing results and percentage of ASVs assigned to the different taxonomic levels can be found in Table S1.

2.6. Quality control

A rarefaction curve was generated using the R-package Ampvis2 (Andersen et al., 2018) to examine the quality of the sequencing (Fig. S1). Based on the rarefaction curve, the sequencing appeared to be of good quality and most of the bacterial diversity seemed to have been uncovered during sequencing. Furthermore, ASVs were observed in the positive control samples and only few or no ASVs were observed in the negative control samples, further supporting the good quality of the sequencing.

2.7. Statistical analyses

Prior to any analysis, locations from the habitat type calcareous grassland were divided into two groups (calcareous grassland and calcareous acidic grassland) based on soil pH. This division was used throughout the data analysis and the present study, giving a total of seven habitat types. The locations in calcareous acidic grassland borders between acidic and calcareous grassland, as grasslands with a pH below 5 are considered acidic in Denmark (Nygaard et al., 2016). All statistical analyses were performed in R v.4.2.1, and a significance level of 0.05 was used unless otherwise stated.

2.7.1. Richness and Shannon's diversity index

Richness was calculated for vegetation and bacteria, as the total number of plant species found within each circle and the total number of ASVs in each sample, respectively. Species richness is the simplest

method for measuring diversity, as it only considers how many species are present (Kiester, 2013). To take into account not only the presence of species but also their abundance, Shannon's diversity index (Shannon, 1948) was calculated as a measure of diversity for vegetation and bacteria. Prior to this, plant species abundance from the three pinpoint frames within each site was summed up. Shannon's diversity index (H) was calculated using the formula

$$1 - \sum p_i \cdot \log(p_i) \quad (1)$$

where p_i is the proportional abundance of species i (or ASV i). Furthermore, Pielou's Evenness (E) (Pielou, 1966) was calculated using the formula:

$$H / \log(S) \quad (2)$$

where H is Shannon's diversity index, and S is the total number of species (or ASVs). Shannon's diversity index and Pielou's Evenness were calculated for each location to compare the alpha diversity between different habitat types. To account for differences in library size, bacterial data was rarefied to 9000 reads prior to alpha diversity analysis but data was not rarefied prior to the other analysis. The R-packages Vegan (Oksanen et al., 2013) and Ampvis2 (Andersen et al., 2018) were used to calculate species/ASV richness, Shannon's diversity index and Pielou's evenness.

2.7.2. Testing for significant differences

To test for significant differences in richness, diversity, and soil properties between habitat types, the lmer function from the lme4 package (Bates et al., 2015) was used to build mixed linear models for each variable. Location was considered as a nested factor to avoid pseudoreplication, and the emmeans package (Lenth et al., 2018) was used to calculate effect sizes and make post hoc pairwise comparisons. If necessary, data was transformed prior to analysis. To account for the multiple pairwise comparisons, Šidák correction was used to adjust the p-values (Šidák, 1967).

2.7.3. Ordination analysis

Unconstrained ordination analysis was used to examine dissimilarity in community composition (beta diversity) between samples/sites for bacteria and vegetation. The R-packages Vegan (Oksanen et al., 2013) and Ampvis2 (Andersen et al., 2018) were used to perform two types of ordination analysis: Principal Coordinates Analysis (PCoA) and non-Metric Multidimensional Scaling Analysis (NMDS) (Legendre and Legendre, 2012), using the Bray-Curtis distance measure (Bray and Curtis, 1957). Abundance data was used, including 113 plant species and 854 ASVs, as ASVs not present in more than 0.1% relative abundance in any sample were removed. No initial data transformation was applied, but sites from the same location were averaged (and summed up) to avoid pseudoreplication.

2.7.4. Heatmap

To examine the 25 bacterial genera with the highest relative abundance in the 52 soil samples, a heatmap was generated using the Ampvis2-package. Samples were grouped by habitat type, and samples from the same habitat type were merged by calculating the mean relative abundance.

2.7.5. PERMANOVA analysis

To evaluate the set of variables best able to explain the community composition of bacteria and vegetation, respectively, the R-package AICcPerm (Corcoran, 2023) was used for model selection based on PERMANOVA-analysis. Location was considered a nested factor to avoid pseudoreplication. The analysis was conducted separately for bacteria and vegetation, as Shannon's diversity index for bacteria was included as a variable in the dataset used to analyse vegetation and vice versa. The following variables were included in the analysis: habitat type, OC, clay content, silt content, sand content, fines (silt + clay content), clay:OC-ratio, pH, EC, SWC, WR, NH_4^+, $NO_3^- + NO_2^-$, and Shannon's diversity index (vegetation/bacteria). All possible models were generated using the full set of variables and Variance Inflation Factor (VIF) was used to avoid multicollinearity by filtering out models with a VIF >6. The remaining models were fitted and Akaike's information criterion with correction for small sample size (AICc) was calculated for each model. Models differing with less than 2 AICc from the best model were evaluated and the best models including one to five variables were selected based on AICc. Furthermore, the analysis were repeated with habitat type excluded as a variable. For vegetation, separate models were made using presence/absence data and abundance data as more species were included in the presence/absence dataset, compared to the abundance dataset, due to differences in sampling area. The analysis of presence/absence data was based on the Jaccard distance measure (Jaccard, 1901) while the Bray-Curtis distance measure (Bray and Curtis, 1957) was used for abundance data.

3. Results

3.1. Soil properties in the habitat types

The average soil texture was dominated by sand across the habitat types except for calcareous grassland, which had the lowest average sand content (48.53 ± 21.79%), followed by wet heath (68.58 ± 20.27%) and dry heath (82.36 ± 6.31%). Similarly, the highest average clay content was found in calcareous grassland (18.43 ± 8.14%), followed by fixed dune (8.13 ± 10.79%) and wet heath (7.04 ± 6.69%). Further, calcareous grassland, fixed dune, and wet heath also exhibited relatively large variations in soil texture compared to the other habitat types (Table 2). For the soil texture, the post hoc pairwise comparisons showed a significant difference between the habitat types for the content of sand ($F_{6, 17} = 4.52$, $p = 0.0062$) and silt ($F_{6, 17} = 5.26$, $p = 0.003$).

The OC also differed significantly across the habitat types ($F_{6, 20} = 10.64$, $p < 0.001$), ranging between 0.27% and 16.61% across all soil samples. The highest average OC was found in wet heath (8.79 ± 5.41%) and dry heath (7.38 ± 3.79%), while the lowest average OC was found in calcareous acidic grassland (1.56 ± 0.62%) and sandy grassland (1.81 ± 1.50%). The clay fraction of soil can stabilize OC through the process of organo-mineral complexation (Lehmann and Kleber, 2015) and a clay:OC ratio of 10 (Dexter et al., 2008) is often used as a threshold to define whether a soil might contain non-complexed amounts of either clay or OC (e.g. de Jonge et al., 2009; Jensen et al., 2017; Schjønning et al., 2012). Almost all the investigated soils had a clay:OC ratio below 10, indicating that they contained non-complexed OC (Fig. 2A).

None of the soil samples from calcareous grassland showed water repellency (Table 2). In sandy grassland and fixed dune, some soil samples were water repellent while all samples showed water repellency in calcareous acidic grassland, acidic grassland, dry heath, and wet heath. Consequently, WR differed significantly between habitat types ($F_{6, 18} = 11.86$, $p < 0.001$). A significant relationship was found between the degree of water repellency and the clay:OC ratio ($F_{1, 49} = 24.47$, $p < 0.001$) (Fig. 2B). Prior to the analysis, one outlier was removed (see Fig. S2 for the analysis with all samples included).

The pH differed significantly between habitat types ($F_{6, 19} = 212.23$, $p < 0.001$), ranging from 4.03 to 8.77 across all habitat types, with calcareous and sandy grasslands exhibiting significantly higher pH values compared to the remaining habitat types. Furthermore, the water content varied markedly across the habitat types, ranging between 1.43% and 80.87% and exhibiting a significant difference in mean values ($F_{6, 20} = 19.47$, $p < 0.001$).

3.2. Alpha diversity of bacteria and vegetation in the habitat types

The highest diversity and richness of bacteria and vegetation was observed in acidic grassland (Table 3). Apart from this similarity,

Table 2
Mean, standard deviation (SD) and range (minimum-maximum) for the soil properties measured on the 52 soil samples. Samples were collected from 19 locations and within each location, samples were collected from two to three different sites per location (giving a total of 52 sites). The locations represent seven habitat types (six to nine replicates (sites) per habitat type, indicated in the table). Means not sharing any letter are significantly different according to the post hoc pairwise comparisons at a significance level of 0.05. For this analysis, location was considered as a nested factor to avoid pseudoreplication. Abbreviations: EC = electrical conductivity, SWC = water content, WR = potential water repellency of soil samples, NH_4^+ = ammonium, $NO_3^- + NO_2^-$ = nitrate + nitrite, OC = organic carbon content.

Mean ±SD	Calc. grass. n = 6		Calc. acidic grass. n = 6		Acidic grass. n = 8		Sandy grass. n = 7		Dry heath n = 8		Wet heath n = 8		Fixed dune n = 9	
	[min max]	Mean ± SD	[min max]	Mean ±SD	[min max]		Mean ± SD	[min max]	Mean ± SD	[min max]	Mean ± SD	[min max]	Mean ± SD	[min max]
Sand (%)	48.53 ± 21.79 **a**	29.04 78.08	90.70 ± 3.73 **ab**	85.06 95.82	84.94 ± 4.15 **ab**	78.49 91.45	91.72 ± 5.40 **b**	82.60 97.34	82.36 ± 6.31 **b**	73.61 90.47	68.58 ± 20.27 **ab**	40.01 92.55	82.88 ± 22.26 **ab**	43.38 98.58
Silt (%)	28.45 ± 16.06 **a**	9.40 46.10	2.73 ± 1.99 **b**	0.00 5.80	3.14 ± 1.38 **b**	1.60 6.20	1.67 ± 1.35 **b**	0.20 3.70	2.11 ± 1.75 **b**	0.00 5.00	9.23 ± 11.17 **ab**	0.70 23.00	5.44 ± 8.18 **b**	0.00 20.10
Clay (%)	18.43 ± 8.14 a	10.10 32.80	3.88 ± 1.59 ab	2.00 6.30	3.46 ± 0.76 ab	2.70 4.90	3.49 ± 2.04 ab	1.60 6.70	2.81 ± 1.05 b	1.40 4.10	7.04 ± 6.69 ab	0.60 15.40	8.13 ± 10.79 ab	0.40 27.00
OC (%)	2.66 ± 1.27 **a**	1.40 4.62	1.56 ± 0.62 **ab**	0.68 2.40	4.91 ± 2.26 abc	2.16 8.24	1.81 ± 1.50 **ab**	0.27 4.06	7.38 ± 3.79 **bc**	2.28 12.73	8.79 ± 5.41 **c**	2.22 16.61	2.06 ± 2.60 **a**	0.35 8.02
WR (mN m^{-1})[a]	71.27 ± 0.00 **a**	71.27 71.27	50.84 ± 5.17 **c**	58.55 45.36	46.93 ± 5.77 **bc**	56.55 40.88	58.62 ± 10.19 **ab**	71.27 45.36	42.82 ± 3.43 **bc**	49.34 39.65	41.94 ± 6.19 **c**	54.80 35.99	53.38 ± 11.96 **bc**	71.27 39.07
pH	8.47 ± 0.10 **a**	8.33 8.59	5.00 ± 0.18 **b**	4.68 5.24	4.70 ± 0.21 **b**	4.42 4.97	8.19 ± 0.49 **a**	7.46 8.77	4.40 ± 0.30 **b**	4.03 4.83	4.49 ± 0.20 **b**	4.30 4.89	4.67 ± 0.31 **b**	4.25 5.10
SWC (%)	31.46 ± 17.65 **ab**	11.11 53.47	5.48 ± 4.41 **cd**	1.43 10.84	17.85 ± 3.74 **abcd**	12.78 23.15	5.56 ± 3.75 **acd**	1.96 10.46	15.18 ± 5.58 **ad**	7.14 22.65	50.48 ± 22.96 **b**	19.29 80.87	8.76 ± 8.55 **c**	1.80 22.90
EC (μS/cm)	555.8 ± 146.9 **a**	375.0 708.0	116.7 ± 25.4 **ab**	92.0 149.0	165.5 ± 44.2 **ab**	86.0 204.0	414.4 ± 264.0 **ab**	191.0 917.0	184.6 ± 65.2 **b**	99.0 271.0	180.1 ± 86.5 **b**	95.0 362.0	116.1 ± 89.6 **b**	38.0 294.0
NH_4^+ (mg/kg)	54.86 ± 42.52 **a**	9.85 108.35	19.59 ± 24.62 **a**	1.96 64.41	115.62 ± 84.53 **ab**	29.66 294.36	31.95 ± 36.46 **a**	4.31 105.43	13.70 ± 12.08 **a**	2.95 40.36	258.40 ± 201.11 **b**	48.35 604.33	17.31 ± 18.95 **a**	1.61 45.05
$NO_3^- + NO_2^-$(mg/kg)	411.58 ± 625.17 **a**	0.00 1476.86	6.22 ± 6.90 **a**	1.37 19.08	21.67 ± 22.39 **a**	0.00 57.47	50.85 ± 64.15 **a**	1.54 154.00	0.33 ± 0.92 **a**	0.00 2.60	66.95 ± 69.92 **a**	9.67 183.82	3.97 ± 3.67 **a**	0.50 9.24

[a] The measure of WR is inverse, i.e. the higher the WR, the lower the surface tension. Thus, in Table 2, the highest degree of water repellency is obtained for the wet heath (35.99 mN m^{-1}).

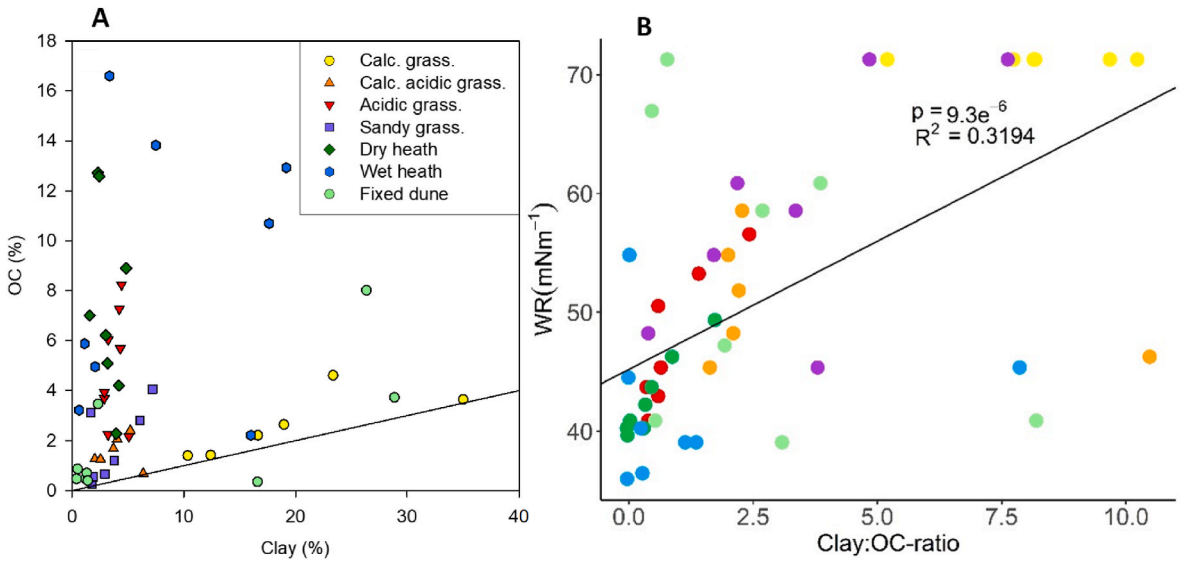

Fig. 2. Plot of organic carbon content (OC) as a function of clay content for the 52 soil samples (A). The clay-to-OC ratio of 10 is indicated by the clay-to-OC saturation line. Water repellency (WR) as a function of clay:OC ratio for 51 soil samples (B). Prior to the regression shown on (B), one outlier was excluded. Samples were collected from 19 locations and within each location, samples were collected from three different sites per location. The locations represent seven habitat types (six to nine replicates (sites) per habitat type). Samples are coloured according to habitat type.

Table 3
Mean, standard deviation (SD) and range (minimum:maximum) for the species/ASV richness (S/ASV), Shannon's diversity index (H) and Pielou's Evenness (E) of the plant and bacterial communities found in the 52 sites/soil samples. Data originates from botanical surveys conducted in 19 locations and soil samples collected within the same 19 locations for amplicon sequencing. Botanical surveys and sample collection were conducted in three different sites per location. The locations represent seven habitat types (six to nine replicates (sites) per habitat type, indicated in the table). Means not sharing any letter are significantly different according to the t-statistic at a significance level of 0.05. For this analysis, location was considered as a nested factor to avoid pseudoreplication. Abbreviations: S = species richness, H = Shannons' diversity index, E = Pielous' evenness, ASV = Amplicon Sequence Variant-richness.

Mean ±SD	Calc. grass. n = 6		Calc. acidic grass. n = 6		Acidic grass. n = 8		Sandy grass. n = 7		Dry heath n = 8		Wet heath n = 8		Fixed dune n = 9	
	[min max]	Mean ±SD	[min max]	Mean ±SD	[min max]	Mean ± SD	[min max]	Mean ± SD	[min max]	Mean ± SD	[min max]	Mean ± SD	[min max]	
Vegetation														
S	19 ±4.22 **abcde**	12 23	14 ±2.14 abc	11 17	22 ±3 **abcde**	20 27	21 ±1.35 **de**	19 23	12 ±8.73 **bd**	5 27	16 ±7.75 **ce**	8 26	11 ±3.86 **a**	6 17
H	1.90 ±0.26 abc	1.54 2.22	1.74 ±0.25 abc	1.46 2.11	2.26 ±0.23 **bc**	1.89 2.55	1.80 ±0.24 abc	1.42 2.09	1.47 ±2.39 **ab**	0.66 2.26	1.88 ±0.36 **c**	1.47 2.39	1.23 ±0.47 **a**	0.64 1.72
E	0.80 ±0.04 **a**	0.74 0.86	0.79 ±0.05 **a**	0.69 0.81	0.86 ±0.04 **a**	0.79 0.90	0.83 ±0.03 **a**	0.78 0.87	0.73 ±0.14 **a**	0.48 0.87	0.85 ±0.05 **a**	0.75 0.90	0.77 ±0.12 **a**	0.58 0.88
Bacteria														
ASV	1289 ±277.83 **a**	785 1582	3933 ±275.49 **bc**	3431 4177	4741 ±285.15 **c**	4237 5096	1566 ±140.85 **a**	1325 1698	4312 ±391.01 **c**	3645 4902	3507 ±874.34 **b**	2116 4631	4027 ±497.93 **bc**	3495 4880
H	6.11 ±0.35 **a**	5.47 6.47	7.81 ±0.12 **bc**	7.59 7.90	8.11 ±0.11 **c**	7.93 8.26	6.39 ±0.23 **a**	6.10 6.66	7.93 ±0.18 **c**	7.65 8.15	7.52 ±0.51 **b**	6.61 8.06	7.84 ±0.19 **bc**	7.63 8.15
E	0.85 ±0.02 **a**	0.82 0.88	0.94 ±0.01 **b**	0.93 0.95	0.96 ±0.01 **b**	0.95 0.97	0.87 ±0.02 **a**	0.84 0.90	0.95 ±0.01 **b**	0.93 0.96	0.92 ±0.03 **b**	0.86 0.95	0.95 ±0.01 **b**	0.93 0.96

different trends were observed for bacteria and vegetation.

For bacteria, a high diversity and richness was observed in the habitat types with low pH (Table 3 and Fig. S3). These differed significantly from the calcareous and sandy grasslands where a lower diversity and richness was observed (Table 3). Shannon's diversity index ($F_{6, 19} = 47.78$, $p < 0.001$), ASV richness ($F_{6, 21} = 49.80$, $p < 0.001$) and evenness ($F_{6, 16} = 28.69$, $p < 0.001$) differed significantly between habitat types. A high diversity of bacteria was observed in most soil samples.

For vegetation, the lowest richness was observed in the heathland habitats (Table 3). The richness was highest in the grasslands, except for one location of wet heath and dry heath, where a high richness was observed (Fig. S4). Plant diversity showed the same trends as richness, but differences among habitat types were less evident due to the high evenness observed in all habitat types (Table 3). Shannon's diversity index ($F_{6, 20} = 9.38$, $p < 0.001$) and species richness ($F_{6, 19} = 15.89$, $p < 0.001$) differed significantly between habitat types, but Pielou's evenness did not ($F_{6, 17} = 2.48$, $p = 0.0653$).

3.3. Community composition and habitat specificity

Habitat-specific communities were observed for both vegetation (Fig. 3A and Fig. S5A) and bacteria (Fig. 3B and Fig. S5B), although some overlaps between habitat types were observed, particularly for bacteria.

For bacteria, the largest difference was found between the habitats with high (calcareous grassland and sandy grassland) and low pH

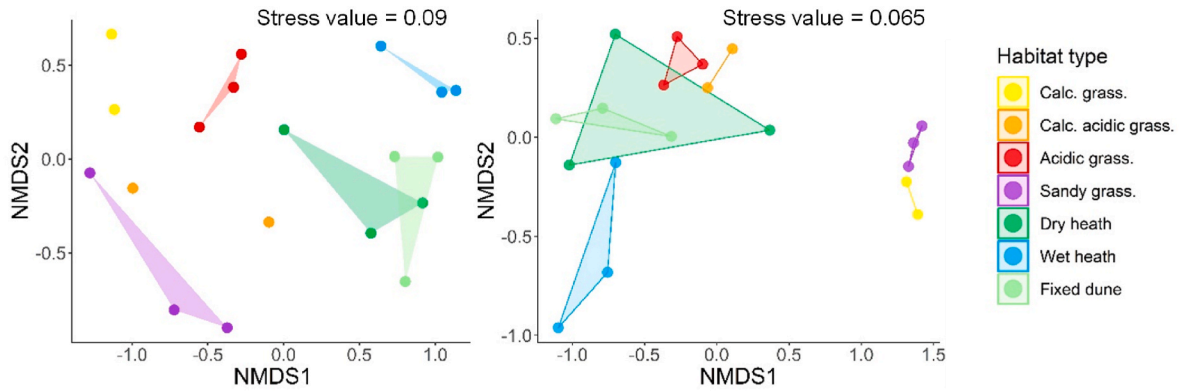

Fig. 3. non-Metric Multidimensional Scaling Analysis (NMDS) based on the Bray-Curtis distance measure (Bray and Curtis, 1957) of (A) 113 plant species and (B) 854 ASVs. Prior to the analysis of (B), ASVs that were not present in more than 0.1% relative abundance in any sample were removed. Data originates from botanical surveys conducted in 19 locations and soil samples collected within the same 19 locations for amplicon sequencing. Botanical surveys and sample collection were conducted in three different sites per location. The locations represent seven habitat types. Six to nine replicates were included per habitat type, but sites from the same location were averaged (and summed up) to avoid pseudoreplication (giving two to three replicates per habitat type). No initial data transformation was applied for either (A) or (B). Samples are coloured according to habitat type.

(calcareous acidic grassland, acidic grassland, dry heath, wet heath, and fixed dune), respectively (Fig. 3B). Similarities in community composition were observed between some habitat types, particularly between dry heath and fixed dune (Fig. 3B and Fig. S5B). This was also observed for vegetation (Fig. 3A). For vegetation, the largest difference was observed between the grassland and heathland habitats. Locations from the same habitat type generally clustered (Fig. 3A and Fig. S5A), with two exceptions: One location from sandy grassland was more like locations from the two types of calcareous grassland one location from dry heath was more similar to the locations from acidic grassland (Fig. 3A).

The difference between the bacterial communities in the habitat types with high and low pH, was also evident among the 25 most abundant bacterial genera (Fig. 4). The most abundant genera in the habitat types with low pH, *Acidothermus*, *Subgroup 2*, and *Roseiarcus*, were not observed in the habitat types with high pH. Similarly, the most

	Calc. grass.	Calc. acidic grass.	Acidic grass.	Sandy grass.	Dry heath	Wet heath	Fixed dune
Actinobacteriota; Acidothermus	0	8.6	9.8	0	13.1	10.1	17
Acidobacteriota; Subgroup_2	0	3.5	5.8	0	8.7	9.6	5.8
Proteobacteria; Roseiarcus	0	2.2	2.6	0	3.3	4.5	5.4
Verrucomicrobiota; Candidatus_Xiphinematobacter	1.8	1.1	3.8	1.6	6.9	0.9	1.2
Actinobacteriota; Conexibacter	0.5	5.1	4	1.1	2	1.4	3.3
WPS-2; WPS-2	0	3.3	4.6	0	3.1	1.8	2.9
Proteobacteria; Bradyrhizobium	1.9	5.2	4.1	2.3	1.3	0.6	1.4
Actinobacteriota; Mycobacterium	2.6	2.3	1.5	3.6	2.2	1	3
Verrucomicrobiota; Candidatus_Udaeobacter	1	4.6	4.4	2.1	1.8	0.9	0.7
Chloroflexi; KD4-96	7.5	0.6	0.2	7.2	0.3	0	0.1
RCP2-54; RCP2-54	0	0.6	1.3	0	3	4.4	3.2
Chloroflexi; AD3	0	2.5	1.6	0	2.8	0.7	4
Acidobacteriota; Vicinamibacteraceae	6.4	0	0	3.8	0.3	0	0
Actinobacteriota; Solirubrobacter	2.9	0.1	0	6	0.1	0	0
Proteobacteria; Acidibacter	0.2	1.4	1.7	0.2	1.1	0.9	0.9
Acidobacteriota; Occallatibacter	0	0.7	0.6	0	0.7	1.5	2.4
Proteobacteria; WD260	0	1.5	1.4	0	1	0.7	1.3
Acidobacteriota; Candidatus_Koribacter	0	0.4	0.5	0	0.5	2.8	1.2
Actinobacteriota; Streptomyces	0.6	0.3	0.2	4.4	0.3	0	0
Acidobacteriota; Candidatus_Solibacter	0.2	1	0.9	0.1	0.5	1.6	0.9
Actinobacteriota; 67-14	1.7	0.3	0.1	3	0.1	0.1	0.3
Planctomycetota; Aquisphaera	0	0.3	0.4	0	0.4	2.5	0.6
Acidobacteriota; RB41	1.4	0	0	3.4	0.1	0	0
Acidobacteriota; Acidipila	0	0.4	0.5	0	1.1	0.7	1.3
Chloroflexi; HSB_OF53-F07	0	0.3	1.3	0	0.6	1	0.6

Fig. 4. Heatmap showing the 25 bacterial genera with the highest relative abundance in the 52 soil samples. Data originates amplicon sequencing of soil samples collected within the 19 locations. Within each location, samples were collected from three different sites. The locations represent seven habitat types (six to nine replicates (sites) per habitat type). Each column shows the sequencing result for the samples within one of the seven habitat types. The identity of bacteria is given as phylum and genera, separated by a semicolon. The numbers show the relative abundance of the bacteria in percentage based on V4 amplicon data (average of all samples within the same habitat type), also indicated with the colour intensity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

abundant genera in the samples from the habitat types with high pH were either not observed in the habitat types with low pH or only found in low abundance. Generally, bacterial communities consisted of few genera with high abundance, while most genera were low-abundant.

3.4. Models explaining community composition

The abundance of vegetation could best be explained by a model including habitat type and EC, but only differed little in AICc compared to the model including habitat type as the only variable (Table 4). Habitat type alone could explain 47% of the variation in community composition (results available here) and was selected as a variable in all models, if not excluded. Excluding habitat type, the model best able to explain plant community composition could explain 25% of the variation and included pH, EC, ammonium content and Shannon's Diversity Index for bacteria (Table 4). pH was included in all models excluding habitat type.

As previously mentioned, separate analyses were conducted for vegetation, using abundance and presence/absence data, respectively. Only the results based on abundance data are shown here. Results based on presence/absence data can be found in Supplementary Materials (Table S2).

Bacterial abundance could best be explained by a model including habitat type and OC, but as seen for vegetation, this model differed little in AICc compared to the model only including habitat type (Table 5). Habitat type alone could explain 46% of the variation in community composition (results available here) and habitat type was included in all models if not excluded, as seen for vegetation. If habitat type was excluded, the best model could explain 37% of the variation and included pH, SWC, OC, and Shannon's diversity index for vegetation (Table 5). As for vegetation, pH was included in all models where habitat type was excluded.

Including habitat type as a variable generally improved the models, seen by a decrease in the AICc, for both vegetation (Table 4) and bacteria (Table 5).

Table 4
The best models explaining the community composition of vegetation (abundance) including or excluding habitat type as a variable. The results are based on a PERMANOVA-analysis where the best models were selected by AICc. The models best explaining community composition using one through five variables are shown. The full set of variables included in the analysis were: habitat type, OC, clay content, silt content, sand content, fines (silt + clay content), clay:OC-ratio, pH, EC, SWC, WR, NH_4^+, NO_3^- + NO_2^-, and Shannon's diversity index for bacteria. All possible models were generated using the full set of variables and Variance Inflation Factor (VIF) was used to avoid multicollinearity by filtering out models with a VIF >6. Data originates from botanical surveys conducted in 19 locations. Furthermore, soil samples were collected within the same 19 locations for amplicon sequencing (to calculate the diversity of bacteria) and analysis of soil properties. Botanical surveys and sample collection were conducted in three different sites per location. The locations represent seven habitat types (six to nine replicates (sites) per habitat type, indicated in the table). Location was considered a nested factor to avoid pseudoreplication. Abbreviations: EC = electrical conductivity, AICc = Akaike's information criterion with correction for small sample size, NH_4^+ = ammonium content, H_{bac} = Shannon's diversity index, bacteria.

Number of variables included	Vegetation, abundance			
	Including habitat type		Excluding habitat type	
	Best model using the number of variables	AICc	Best model using the number of variables	AICc
1	Bray Curtis distance ~ Habitat type	−75.690	–	–
2	Bray Curtis distance ~ Habitat type + EC	−75.760	Bray Curtis distance ~ pH + H_{bac}	−59.392
3	Bray Curtis distance ~ Habitat type + EC + NH_4^+	−74.463	Bray Curtis distance ~ pH + EC + H_{bac}	−60.196
4	–	–	Bray Curtis distance ~ pH + EC + NH_4^+ + H_{bac}	−60.587

Table 5
The best models explaining the community composition of bacteria (abundance) including or excluding habitat type as a variable. The results are based on a PERMANOVA-analysis where the best models were selected by AICc. The models best explaining community composition using one through five variables are shown. The full set of variables included in the analysis were: habitat type, OC, clay content, silt content, sand content, fines (silt + clay content), clay:OC-ratio, pH, EC, SWC, WR, NH_4^+, NO_3^- + NO_2^-, and Shannon's diversity index for vegetation. All possible models were generated using the full set of variables and Variance Inflation Factor (VIF) was used to avoid multicollinearity by filtering out models with a VIF >6. Data originates from soil samples collected within 19 locations for amplicon sequencing and analysis of soil properties. Furthermore, data from botanical surveys conducted in the same 19 locations were used to calculate the diversity of vegetation. Sample collection and botanical surveys were conducted in three different sites per location. The locations represent seven habitat types (six to nine replicates (sites) per habitat type, indicated in the table). Location was considered a nested factor to avoid pseudoreplication. Abbreviations: OC = organic carbon content, SWC = soil water content, AICc = Akaike's information criterion with correction for small sample size, H_{veg} = Shannon's diversity index, vegetation.

Number of variables included	Bacteria, abundance			
	Including habitat type		Excluding habitat type	
	Best model using the number of variables	AICc	Best model using the number of variables	AICc
1	Bray Curtis distance ~ Habitat type	−85.730	–	–
2	Bray Curtis distance ~ Habitat type + OC	−86.086	Bray Curtis distance ~ pH + SWC	−81.083
3	Bray Curtis distance ~ Habitat type + OC + H_{veg}	−85.789	Bray Curtis distance ~ pH + SWC + H_{veg}	−81.568
4	–	–	Bray Curtis distance ~ pH + SWC + OC + H_{veg}	−82.119

4. Discussion

4.1. Representativity of the habitat types and differences in soil properties

In general, the habitat types in the present study were found to be representative as the meassured soil properties were in line with the national average (Nygaard et al., 2016) and the natural variation in the descriptions of the habitat types (Commission, 2013; Natura, 2016). Compared to the average pH values for the habitat types in Denmark (Nygaard et al., 2016), the pH values in the present study were generally higher but still within the expected range. As expected, the highest water content was found in wet heath, but surprisingly, this was not significantly different from the water content measured in calcareous or acidic grasslands. While the high water content of the calcareous grasslands may be attributed to their relatively fine-grained particle size distribution, the high water content of the coarse-grained acidic grasslands is probably a reflection of precipitation during the sampling period (Zheng et al., 2015) or a wetter moisture regime.

The clay fraction of soil can stabilize OC through the process of organo-mineral complexation (Lehmann and Kleber, 2015). While the capacity of clay to form organo-mineral complexes depends on, e.g., the type of OC, the clay mineralogy, and the specific surface area (Kleber et al., 2015), Dexter et al. (2008) found that 8–10 g of clay has the capacity to complex 1 g of OC. Consequently, a clay:OC ratio of 10 is often

used as a threshold to define whether a soil might contain non-complexed amounts of either clay or OC (e.g. de Jonge et al., 2009; Jensen et al., 2017; Schjønning et al., 2012). Almost all the investigated soils had a clay:OC ratio below 10, indicating that they contained non-complexed OC available for the bacterial communities. The clay:OC ratio of the specific soil samples, could to some degree explain that some soil samples were water repellent and others were not, as Weber et al. (2021) found that water repellency after 60 °C pre-treatment ceased at clay:OC ratios above 2. Water repellency is often associated with negative effects on crop production, flood risk, etc. (Dekker and Ritsema, 1994; Doerr et al., 2000), but in a study by Seaton et al. (2019), water repellency is suggested to be a response to stress and increase the resilience of ecosystems. Further studies examining this response are necessary, as it is still debated whether water repellency is driven by the biological community structure or vice versa (Lozano et al., 2014; Seaton et al., 2019). As water repellency was observed in most habitat types in the present study, it could be an interesting parameter to include in the future monitoring of the European habitat types. This is further emphasized by the findings by Seaton et al. (2019), as water repellency may mitigate the negative effects of water stress on biological communities.

4.2. Differences in alpha diversity of bacteria and vegetation

A high diversity of bacteria was expected as soil samples generally contain a high diversity of microorganisms, including bacteria (Thompson et al., 2017), which has been shown in surveys covering multiple environments worldwide (e.g., Tedersoo et al., 2014; Davison et al., 2015; Delgado-Baquerizo et al., 2018). The high diversity observed in the grassland habitat types in the present study, is in line with other studies examining grasslands, for example the study by Delgado-Baquerizo and Eldridge (2019) where the highest diversity of bacteria was found in samples from grassland, compared to forests (Delgado-Baquerizo and Eldridge, 2019). In a study by George et al. (2019), a significantly lower diversity of bacteria was observed in heathland compared to acidic grassland. This contrasts with the observations in the present study, but different types of heathlands were grouped together in the study by George et al. (2019), making direct comparisons to the present study difficult. Several studies have shown that bacterial richness is affected by pH (Delgado-Baquerizo and Eldridge, 2019; Fierer and Jackson, 2006; Kaiser et al., 2016; Lauber et al., 2009). Richness is highest at pH-values close to neutral, while it declines as the soil turns more acidic or alkaline (Delgado-Baquerizo and Eldridge, 2019; Fierer and Jackson, 2006; Thompson et al., 2017; Wu et al., 2017). This partially contrast with the result from the present study, as the highest richness was found in samples with low pH compared to the samples with a pH-value close to neutral. A possible explanation for this could be that other properties than pH affects the bacterial communities. In sandy grassland a possible explanation could be the dry conditions, as this is a xeric habitat type and therefore a stressful environment. The opposite could explain the low richness observed in some of the samples from wet heath. Bacteria present in the soil are affected by the soil water content (Bahram et al., 2018; Bickel et al., 2019; Bickel and Or, 2020) as this influences available microhabitats, the mobility of the bacteria and thereby interactions (Bickel et al., 2019; Bickel and Or, 2020). Based on the results in this paper, the habitat types with a high or low water content and/or high pH seem to represent stressful environments and may therefore support a lower diversity than the other habitat types.

Differences in plant species richness and diversity were expected based on the descriptions of the habitat types (Commission, 2013; Habitatnøgle, 2016; Natura, 2016). The habitat types comprise naturally species-rich and species-poor plant communities, which was also observed in the present study: the highest plant diversity was observed in the grasslands, while the heathlands were less diverse. In general, the variation in plant diversity was in line with the natural variation in the descriptions of the habitat types (Commission, 2013; Natura, 2016). The diversity of vegetation is presumed to affect the diversity of soil living bacteria, as a higher plant diversity leads to a more diverse mix of exudates and litter, thereby increasing the diversity of resources available for bacteria (Hooper et al., 2000; Van Der Putten, 2017; Wardle, 2006). This was not reflected in the results in the present study, as no clear-cut correlation between the diversity of vegetation and bacteria was observed in the present paper. Several studies found positive correlations between the diversity of vegetation and bacteria (Eisenhauer et al., 2011; Liu et al., 2020; Milcu et al., 2013), but conflicting results have been reported as well (Millard and Singh, 2010; Prober et al., 2015; Wardle, 2006).

4.3. Habitat-specific community composition and explanatory variables

The variations in plant community composition within the same habitat type seen in the present study align with previous studies of the Danish grassland vegetation (Bruun and Ejrnæs, 2000). Differences in management and geographical location could explain variations in community composition within the same habitat type.

Habitat specificity was less evident for bacteria compared to vegetation. Despite this, some degree of habitat specificity was still observed for bacteria as samples from the same habitat type formed clusters. In a meta-analysis by Tripathi et al. (2018), a more homogeneous composition of bacterial communities was seen in soil samples with extremely high or low pH values, because the extreme environmental conditions meant that the composition of the bacterial communities was less random (Tripathi et al., 2018). This may explain the small variation within the bacterial communities observed in samples from sandy grassland and calcareous grassland, as these bacterial communities may consist of ASVs adapted to the stressful conditions of high pH and low water content. Furthermore, it could explain the similarities in bacterial communities found in the two habitat types.

One of the dry heath locations differed from the other locations of that habitat type. For vegetation, this is in line with observations made during the fieldwork, as a low cover of dwarf shrubs and a higher cover of grasses/herbs was observed in this location compared to the other locations of the same habitat type. This can explain why the plant communities in this location were more like those found in acidic grassland. Interestingly, the same could be observed for the bacterial community in the same location, which could indicate that the plant community influences the bacterial community. This was also observed in a study by Boeddinghaus et al. (2019), who concluded that changes in the plant community had an impact on the microbial communities on both the long and short term (Boeddinghaus et al., 2019). Furthermore, the results in the present paper are in line with the findings of Prober et al. (2015), who found a correlation between the beta diversity of vegetation and bacteria, but not the alpha diversity.

As observed in other studies (Delgado-Baquerizo et al., 2018; Janssen, 2006), the bacterial communities were dominated by few genera with a high abundance. Proteobacteria, Actinobacteria and Acidobacteriota were found to be abundant in the studies by Delgado-Baquerizo et al. (2018) and George et al. (2019), across ecosystems and continents. To the knowledge of the authors, little is known about the microbial communities in the habitat types examined in the present study. This is partly because only a small fraction of the microbial communities in soil has been described (Dance, 2020; Delgado-Baquerizo et al., 2018) but also because most studies examining the soil microbiome have used coarser resolutions of habitat types. The findings in the present paper emphasizes the need for national and international projects to increase the current knowledge of terrestrial bacterial communities using a finer resolution of habitat types.

For vegetation, it was not surprising that habitat type was selected at the best explanatory variables, as the habitat types are defined based on the plant communities (Commission, 2013). But the fact that habitat type was the variable best able to explain the variation in community

composition of bacteria suggests that the habitat types feature different combinations of soil properties that shapes the bacterial communities in the habitat types differently. Furthermore, this also suggests that bacterial communities could be implemented in the current monitoring of habitat types. That pH and water content are important variables if habitat type is excluded, is in line with previous studies showing that soil properties, especially pH, shape the bacterial communities (e.g., Chu et al., 2020; Delgado-Baquerizo et al., 2018; George et al., 2019).

4.4. Microbial communities in monitoring and management

Additional studies examining the habitat specificity of bacteria will be necessary, considering the number of samples and habitat types in the present study, but the results presented here are promising. The habitat types do not constitute fully defined units. As Bruun and Ejrnæs (2000) emphasized, a natural variation in plant communities within the defined habitat types is to be expected (Bruun and Ejrnæs, 2000). Based on the results from the present study, this also applies to bacterial communities. This variation does not necessarily make the habitat types less valid. The definition of habitat types is needed in relation to the legislation, monitoring, and management of natural areas (Chytrý et al., 2020; Jung et al., 2020; Kallimanis et al., 2013), of which the Habitats Directive is an example.

Except for forest ecosystems (Xu et al., 2020; Dhyani et al., 2019), limited research has been made into including the microbial communities as indicators in management or monitoring. There has been a growing interest in using microbial communities as indicators of pollution, soil health or ecosystems (Ma et al., 2022). Despite this interest, no previous studies have, to the knowledge of the authors, examined the possibility of using a part of the soil microbiome as indicators of habitat types or in monitoring at the fine resolution of habitat types used in the present paper, as similar types of habitats, for example different types of grasslands, are often grouped. One of the few studies examining the possibility of implementing microbial communities into an existing survey based on vegetation and soil properties was carried out by Khassali et al. (2020). In this study, significant differences in the microbial communities were found when comparing two carob habitats, which differed according to plant composition, diversity, and soil properties. The microbial community was shown to be a relevant addition to current surveys, only focusing on soil properties and vegetation (Khassali et al., 2020). Considering the global biodiversity crisis, it is highly relevant to include both 'micro' and 'macro' organisms in legislation aiming to protect and preserve natural areas as well as in the management of these areas (Cameron et al., 2019; Parker, 2010). Microorganisms should be included, as they are essential for several ecosystem functions at the global level (Chu et al., 2020; George et al., 2019; Jansson and Hofmockel, 2020) and in maintaining a healthy soil. The need to include microbial communities is further supported by the fact that belowground diversity is crucial for the functioning of aboveground diversity (Bardgett & Van Der Putten, 2014). By not including the microorganisms, we could be overlooking important drivers of change in the habitat types. Furthermore, changes in the microbial community composition are not possible to detect if no baseline knowledge is available. This emphasizes the need for national and international projects to increase the current knowledge and improve the current databases. Additionally, microorganisms respond faster to changes in the environment compared to vegetation (Fierer et al., 2021; Lauber et al., 2013) which makes them ideal to include in the monitoring and assessment of habitat types.

5. Conclusion

- The habitat types in the present study were found to be representative as the soil properties were in line with the national average and the natural variation in the descriptions of the habitat types.
- No clear-cut correlation was found between the alpha diversity of vegetation and bacteria.
- Habitat-specific communities were observed for both vegetation and bacteria: For vegetation, the difference between grassland and heathland was most evident, while the largest difference was found between the habitats with high and low pH for bacteria.
- Habitat type alone could explain 46% of the variation in community composition for bacteria and 47% for vegetation.
- There is a need for baseline knowledge about the bacterial community composition in the habitat types, as little is known about this.
- Bacteria could possibly be included in the Habitats Directive. This could aid the identification of habitat types and improve the assessment.

The results from the present study show that it could be possible to include bacteria in the habitats directive. Further work is needed to verify the results of this study. Firstly, the results should be verified using larger datasets due to the small number of samples in the present study. Future studies should also include locations from several countries to see if the results can be upscaled to a larger geographical scale. Furthermore, only a fraction of the habitat types included in the Habitats Directive were examined in the present study, and further studies should examine if the results from the present study are also reflected in other habitat types. Finally, the present study only included bacteria, but future studies should explore the possibility of including a larger part of the microbial community such as fungi and protozoa.

Author contribution statement

Anne-Cathrine Storgaard Danielsen: Conceptualization; Methodology; Software; Formal analysis; Investigation; Data Curation; Writing - Original Draft; Writing - Review & Editing; Visualization, Per Halkjær Nielsen: Conceptualization; Methodology; Resources; Writing - Review & Editing; Visualization; Supervision; Project administration; Funding acquisition, Cecilie Hermansen: Software; Formal analysis; Investigation; Writing - Review & Editing; Visualization; Supervision, Peter Lystbæk Weber: Writing - Review & Editing; Visualization; Supervision, Lis Wollesen de Jonge: Conceptualization; Methodology; Resources; Writing - Review & Editing; Visualization; Supervision; Project administration; Funding acquisition, Vibeke Rudkjøbing Jørgensen: Investigation; Data Curation; Writing - Review & Editing; Supervision, Mogens Humlekrog Greve: Conceptualization; Resources; Writing - Review & Editing; Supervision, Derek Corcoran: Software; Formal analysis; Data Curation; Writing - Review & Editing, Morten Kam Dahl Dueholm: Writing - Review & Editing; Visualization; Supervision, Dan Bruhn: Conceptualization; Methodology; Resources; Writing - Review & Editing; Visualization; Supervision; Project administration.

Funding

This work was supported by SustainScapes – Center for Sustainable Landscapes under Global Change [NOVO grant NNF20OC0059595] and by Poul Due Jensen Foundation (Microflora Danica).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests Anne-Cathrine Storgaard Danielsen reports financial support was provided by Novo Nordisk Foundation. Per Halkjær Nielsen reports financial support was provided by Poul Due Jensen Foundation.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank the laboratory technicians Helle Blendstrup, Sofie Albrekt Hansen, Palle Jørgensen, and Anette Ladefoged Kromann for assisting with the laboratory analysis. We would also like to thank Lisette Thomsen for her guidance and assistance during the molecular work. Further, we would like to thank Kasper Skytte Andersen for his comments during the data analysis and Maarit Mäenpää for the comments and guidance with statistics. Last but not least, we would like to thank the landowners for giving access to and assisting with knowledge about the locations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.118677.

References

Andermann, T., Antonelli, A., Barrett, R.L., Silvestro, D., 2022. Estimating alpha, beta, and gamma diversity through deep learning. Front. Plant Sci. 13.
Andersen, K.S., Kirkegaard, R.H., Karst, S.M., Albertsen, M., 2018. ampvis2: an R Package to Analyse and Visualise 16S rRNA Amplicon Data. BioRxiv, 299537.
Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. 75 (2), 129–137. https://doi.org/10.3354/ame01753.
Areco, M.M., Salomone, V.N., Afonso, M.D.S., 2021. Ulva lactuca: a bioindicator for anthropogenic contamination and its environmental remediation capacity. Mar. Environ. Res. 171, 105468 https://doi.org/10.1016/j.marenvres.2021.105468.
Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., Bengtsson-Palme, J., Anslan, S., Coelho, L.P., Harend, H., 2018. Structure and function of the global topsoil microbiome. Nature 560 (7717), 233–237.
Bardgett, R.D., Van Der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515 (7528), 505–511.
Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.
Bickel, S., Chen, X., Papritz, A., Or, D., 2019. A hierarchy of environmental covariates control the global biogeography of soil bacterial richness. Sci. Rep. 9 (1), 1–10.
Bickel, S., Or, D., 2020. Soil bacterial diversity mediated by microscale aqueous-phase processes across biomes. Nat. Commun. 11 (1), 1–9.
Boeddinghaus, R.S., Marhan, S., Berner, D., Boch, S., Fischer, M., Hölzel, N., Kattge, J., Klaus, V.H., Kleinebecker, T., Oelmann, Y., 2019. Plant functional trait shifts explain concurrent changes in the structure and function of grassland soil microbial communities. J. Ecol. 107 (5), 2197–2210.
Bongaarts, J., 2019. IPBES, 2019. Summary for Policymakers of the Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Wiley Online Library.
Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27 (4), 326–349.
Bruun, H.H., Ejrnæs, R., 2000. Classification of dry grassland vegetation in Denmark. J. Veg. Sci. 11 (4), 585–596.
Buchwald, E., 2000. Danske naturtyper: det europæiske NATURA 2000 netværk. Miljø-og energiministeriet, Skov og naturstyrelsen 91.
Cameron, E.K., Martins, I.S., Lavelle, P., Mathieu, J., Tedersoo, L., Bahram, M., Gottschall, F., Guerra, C.A., Hines, J., Patoine, G., 2019. Global mismatches in aboveground and belowground biodiversity. Conserv. Biol. 33 (5), 1187–1192.
Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W., Classen, A.T., 2019. Scientists' warning to humanity: microorganisms and climate change. Nat. Rev. Microbiol. 17 (9), 569–586.
Chu, H., Gao, G.-F., Ma, Y., Fan, K., Delgado-Baquerizo, M., 2020. Soil microbial biogeography in a changing world: recent advances and future perspectives. mSystems 5 (2), e00803-00819.
Chytrý, M., Tichý, L., Hennekens, S.M., Knollová, I., Janssen, J.A., Rodwell, J.S., Peterka, T., Marcenò, C., Landucci, F., Danihelka, J., 2020. EUNIS Habitat Classification: expert system, characteristic species combinations and distribution maps of European habitats. Appl. Veg. Sci. 23 (4), 648–675.
Commission, E., 2013. Interpretation Manual of European Union Habitats. https://ec.europa.eu/environment/nature/legislation/habitatsdirective/docs/Int_Manual_EU28.pdf.
Corcoran, D.. AICcPermanova: Model Selection of PERMANOVA Models Using AICc. Zenodo. https://doi.org/10.5281/zenodo.7816863.
Dance, A., 2020. The search for microbial dark matter. Nature 582 (7811), 301–304.
Davison, J., Moora, M., Öpik, M., Adholeya, A., Ainsaar, L., Bâ, A., Burla, S., Diedhiou, A., Hiiesalu, I., Jairus, T., 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349 (6251), 970–973.
de Jonge, L., Jacobsen, O., Møldrup, P., 1999. Soil water repellency: effects of water content, temperature, and particle size. Soil Sci. Soc. Am. J. 63 (3), 437–442.
de Jonge, L.W., Moldrup, P., Schjønning, P., 2009. Soil Infrastructure, Interfaces & Translocation Processes in Inner Space ("Soil-it-is"): towards a road map for the constraints and crossroads of soil architecture and biophysical processes. Hydrol. Earth Syst. Sci. 13 (8), 1485–1502. https://doi.org/10.5194/hess-13-1485-2009.
de Jonge, L.W., Møldrup, P., Jacobsen, O.H., 2007. Soil-water content dependency of water repellency in soils. Soil Sci. 172 (8), 577–588.
Dekker, L.W., Ritsema, C.J., 1994. How water moves in a water repellent sandy soil: 1. Potential and actual water repellency. Water Resour. Res. 30 (9), 2507–2517.
Delgado-Baquerizo, M., Eldridge, D.J., 2019. Cross-biome drivers of soil bacterial alpha diversity on a worldwide scale. Ecosystems 22 (6), 1220–1231.
Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D. J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018. A global atlas of the dominant bacteria found in soil. Science 359 (6373), 320–325.
Dexter, A., Richard, G., Arrouays, D., Czyż, E., Jolivet, C., Duval, O., 2008. Complexed organic matter controls soil physical properties. Geoderma 144, 620–627.
Dhyani, A., Jain, R., Pandey, A., 2019. Contribution of root-associated microbial communities on soil quality of Oak and Pine forests in the Himalayan ecosystem. Trop. Ecol. 60, 271–280. https://doi.org/10.1007/s42965-019-00031-2.
Doerr, S.H., Shakesby, R., Walsh, R., 2000. Soil water repellency: its causes, characteristics and hydro-geomorphological significance. Earth Sci. Rev. 51 (1–4), 33–65.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26 (19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.
Eisenhauer, N., Milcu, A., Sabais, A.C., Bessler, H., Brenner, J., Engels, C., Klarner, B., Maraun, M., Partsch, S., Roscher, C., 2011. Plant diversity surpasses plant functional groups and plant productivity as driver of soil biota in the long term. PLoS One 6 (1), e16055.
Ejrnæs, R., 2011. *Danmarks biodiversitet 2010 : status, udvikling og trusler* (1. oplag. In: Danmarks Miljøundersøgelser.
Faurholdt, N., Schou, J.C., 2012. *Danmarks Skærmplanter* (1. Oplag. BFN.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. USA 103 (3), 626–631.
Fierer, N., Wood, S.A., de Mesquita, C.P.B., 2021. How microbes can, and cannot, be used to assess soil health. Soil Biol. Biochem. 153, 108111.
Gee, G.W., Or, D., 2002. 2.4 Particle-size analysis. Methods of soil analysis: Part 4 physical methods 5, 255–293.
George, P.B., Lallias, D., Creer, S., Seaton, F.M., Kenny, J.G., Eccles, R.M., Griffiths, R.I., Lebron, I., Emmett, B.A., Robinson, D.A., 2019. Divergent national-scale trends of microbial and animal biodiversity revealed across diverse temperate soil ecosystems. Nat. Commun. 10 (1), 1–11.
Grau-Andrés, R., Davies, G.M., Rey-Sanchez, C., Slater, J., 2019. Bryophyte community composition and diversity are indicators of hydrochemical and ecological gradients in temperate kettle hole mires in Ohio, USA. Mires Peat 24 (37), 1–15.
Habitatnøgle, 2016. Nøgle Til Identifikation Af Danske Naturtyper På Habitatdirektivet. Retrieved from. https://mst.dk/media/128610/habitat-key-ver105_opdatering-2016.pdf.
Hermansen, C., Møldrup, P., Müller, K., Jensen, P.W., van den Dijssel, C., Jeyakumar, P., de Jonge, L.W., 2019. Organic carbon content controls the severity of water repellency and the critical moisture level across New Zealand pasture soils. Geoderma 338, 281–290.
Hooper, D.U., Bignell, D.E., Brown, V.K., Brussard, L., Dangerfield, J.M., Wall, D.H., Wardle, D.A., Coleman, D.C., Giller, K.E., Lavelle, P., 2000. Interactions between Aboveground and Belowground Biodiversity in Terrestrial Ecosystems: patterns, Mechanisms, and Feedbacks: we assess the evidence for correlation between aboveground and belowground diversity and conclude that a variety of mechanisms could lead to positive, negative, or no relationship—depending on the strength and type of interactions among species. Bioscience 50 (12), 1049–1061.
Jaccard, P., 1901. Étude comparative de la distribution florale dans une portion des Alpes et des Jura. Bull. Soc. Vaudoise Sci. Nat. 37, 547–579.
Janssen, P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Appl. Environ. Microbiol. 72 (3), 1719–1728.
Jansson, J.K., Hofmockel, K.S., 2020. Soil microbiomes and climate change. Nat. Rev. Microbiol. 18 (1), 35–46.
Jensen, J.L., Schjønning, P., Christensen, B.T., Munkholm, L.J., 2017. Suboptimal fertilisation compromises soil physical properties of a hard-setting sandy loam. Soil Res. 55 (4), 332–340. https://doi.org/10.1071/SR16218.
Jung, M., Dahal, P.R., Butchart, S.H., Donald, P.F., De Lamo, X., Lesiv, M., Kapos, V., Rondinini, C., Visconti, P., 2020. A global map of terrestrial habitat types. Sci. Data 7 (1), 1–8.
Kaiser, K., Wemheuer, B., Korolkow, V., Wemheuer, F., Nacke, H., Schöning, I., Schrumpf, M., Daniel, R., 2016. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. Sci. Rep. 6 (1), 1–12.
Kallimanis, A.S., Dimopoulos, P., Tomaselli, V., Honrado, J., Lucas, R., Marangi, C., Blonda, P., 2013. Comparing Habitat Classification Schemes for Assessing Landscape Diversity.
Karst, S.M., Albertsen, M., Kirkegaard, R.H., Dueholm, M.S., Nielsen, P.H., 2016. Molecular methods. In: van Loosdrecht, M.C.M., Nielsen, P.H., Lopez-Vazquez, C.M., Brdjanovic, D. (Eds.), Experimental Methods in Wastewater Treatment. IWA Publishing, pp. 285–324. https://doi.org/10.2166/9781780404752.
Khassali, H., Baumel, A., Mahé, F., Tournier, E., Tisseyre, P., Prin, Y., Ouahmane, L., Sanguin, H., 2020. The belowground bacterial and fungal communities differed in their significance as microbial indicator of Moroccan carob habitats. Ecol. Indicat. 114, 106341.
Kiester, A.R., 2013. Species diversity, overview. Encyclopedia of Biodiversity (Second Edition). 6 706–714. https://doi.org/10.1016/B978-0-12-384719-5.00133-7.

King, P., 1981. Comparison of methods for measuring severity of water repellence of sandy soils and assessment of some factors that affect its measurement. Soil Res. 19 (3), 275–285.
Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P.S., 2015. Chapter one - mineral–organic associations: formation, properties, and relevance in soil environments. In: Sparks, D.L. (Ed.), Advances in Agronomy, vol. 130. Academic Press, pp. 1–140. https://doi.org/10.1016/bs.agron.2014.10.005.
Kuramae, E.E., Yergeau, E., Wong, L.C., Pijl, A.S., van Veen, J.A., Kowalchuk, G.A., 2012. Soil characteristics more strongly influence soil bacterial communities than land-use type. FEMS Microbiol. Ecol. 79 (1), 12–24.
Ladin, Z.S., Ferrell, B., Dums, J.T., Moore, R.M., Levia, D.F., Shriver, W.G., D'Amico, V., Trammell, T.L., Setubal, J.C., Wommack, K.E., 2021. Assessing the efficacy of eDNA metabarcoding for measuring microbial biodiversity within forest ecosystems. Sci. Rep. 11 (1), 1–14.
Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75 (15), 5111–5120.
Lauber, C.L., Ramirez, K.S., Aanderud, Z., Lennon, J., Fierer, N., 2013. Temporal variability in soil microbial communities across land-use types. ISME J. 7 (8), 1641–1650.
Legendre, P., Legendre, L., 2012. Numerical Ecology. Elsevier.
Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528 (7580), 60–68. https://doi.org/10.1038/nature16069.
Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2018. Emmeans: estimated marginal means, aka least-squares means. R package version 1 (1), 3.
Levy, E.B., Madden, E.A., 1933. The point method for pasture analysis. NZJ Agri 46, 267–279.
Liu, L., Zhu, K., Wurzburger, N., Zhang, J., 2020. Relationships between plant diversity and soil microbial diversity vary across taxonomic groups and spatial scales. Ecosphere 11 (1), e02999.
Lozano, E., García-Orenes, F., Bárcenas-Moreno, G., Jiménez-Pinilla, P., Mataix-Solera, J., Arcenegui, V., Morugán-Coronado, A., Mataix-Beneyto, J., 2014. Relationships between soil water repellency and microbial community composition under different plant species in a Mediterranean semiarid forest. J. Hydrol. Hydromechanics 62 (2), 101.
Ma, F., Wang, C., Zhang, Y., Chen, J., Xie, R., Sun, Z., 2022. Development of microbial indicators in ecological systems. Int. J. Environ. Res. Publ. Health 19 (21). https://doi.org/10.3390/ijerph192113888.
Martínez-Zavala, L., Jordán-López, A., 2009. Influence of different plant species on water repellency in Mediterranean heathland soils. Catena 76 (3), 215–223.
Milcu, A., Allan, E., Roscher, C., Jenkins, T., Meyer, S.T., Flynn, D., Bessler, H., Buscot, F., Engels, C., Gubsch, M., 2013. Functionally and phylogenetically diverse plant communities key to soil biota. Ecology 94 (8), 1878–1885.
Millard, P., Singh, B.K., 2010. Does grassland vegetation drive soil microbial diversity? Nutrient Cycl. Agroecosyst. 88 (2), 147–158.
Mossberg, B., Stenberg, L., 2014. *Den Nye Nordiske Flora* (2. Udgave. 1. Oplag. Gyldendal.
Natura, 2016. Beskrivelse Af Danske Naturtyper Omfattet Af Habitatdirektivet (NATURA 2000 Typer). Retrieved from. https://mst.dk/media/128611/habitatbeskrivelser-2016-ver-105.pdf.
Naturdata, 2020. Naturdata Miljøportal. https://naturdata.miljoeportal.dk.
Nygaard, B., Damgaard, C., Nielsen, K.E., Bladt, J., Ejrnæs, R., 2016. Terrestriske Naturtyper 2004-2015.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan'. Community ecology package, version 2 (9), 1–295.
Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18 (5), 1403–1414. https://doi.org/10.1111/1462-2920.13023.
Parker, S.S., 2010. Buried treasure: soil biodiversity and conservation. Biodivers. Conserv. 19 (13), 3743–3756.
Pielou, E.C., 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. 13, 131–144.
Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., Lind, E.M., Seabloom, E.W., Adler, P.B., Bakker, J.D., 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecol. Lett. 18 (1), 85–95.
Qiagen, 2019. DNeasy 96 PowerSoil Pro QIAcube HT Kit Handbook.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), D590–D596. https://doi.org/10.1093/nar/gks1219.
Roy, J.L., McGill, W.B., 2002. Assessing soil water repellency using the molarity of ethanol droplet (MED) test. Soil Sci. 167 (2), 83–97. https://journals.lww.com/soilsci/Fulltext/2002/02000/ASSESSING_SOIL_WATER_REPELLENCY_USING_THE_MOLARITY.1.aspx.
Sanchez-Hernandez, J.C., 2006. Earthworm biomarkers in ecological risk assessment. Rev. Environ. Contam. Toxicol. 188, 85–126.
Sándor, R., Iovino, M., Lichner, L., Alagna, V., Forster, D., Fraser, M., Kollár, J., Šurda, P., Nagy, V., Szabó, A., 2021. Impact of climate, soil properties and grassland cover on soil water repellency. Geoderma 383, 114780.
Schjønning, P., de Jonge, L.W., Munkholm, L.J., Moldrup, P., Christensen, B.T., Olesen, J. E., 2012. Clay dispersibility and soil friability—testing the soil clay-to-carbon saturation concept. Vadose Zone J. 11 (1) https://doi.org/10.2136/vzj2011.0067.
Schou, J.C., 2006. *Danske Halvgræsser* (2. Reviderede Udgave/Redaktion Jens Christian Schou. BFN [1. oplag].
Schou, J.C., Wind, P., Lægaard, S., 2010. Danmarks Siv Og Frytler. BFN.
Schou, J.C., Wind, P., Lægaard, S., 2014. *Danmarks Græsser* (2. Reviderede Udgave. BFN.
Seaton, F.M., Jones, D.L., Creer, S., George, P.B., Smart, S.M., Lebron, I., Barrett, G., Emmett, B.A., Robinson, D.A., 2019. Plant and soil communities are associated with the response of soil water repellency to environmental stress. Sci. Total Environ. 687, 929–938.
Seberg, O., Barfod, A.S., Frederiksen, S., Rasmussen, F.N., 2012. *Dansk Flora* (2. Udgave. 1. Oplag. Gyldendal.
Shannon, C.E., 1948. A mathematical theory of communication. The Bell system technical journal 27 (3), 379–423.
Šidák, Z., 1967. Rectangular confidence regions for the means of multivariate normal distributions. J. Am. Stat. Assoc. 62 (318), 626–633.
Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., 2014. Global diversity and geography of soil fungi. Science 346 (6213), 1256688.
Thaler, D.S., 2021. Is global microbial biodiversity increasing, decreasing, or staying the same? Frontiers in Ecology and Evolution 9, 565649.
Theron, K.J., Pryke, J.S., Samways, M.J., 2022. Identifying managerial legacies within conservation corridors using remote sensing and grasshoppers as bioindicators. Ecol. Appl. 32 (1).
Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., Prill, R.J., Tripathi, A., Gibbons, S.M., Ackermann, G., Navas-Molina, J.A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J.T., Mirarab, S., Zech Xu, Z., Jiang, L., 2017. The earth microbiome project, C. A communal catalogue reveals Earth's multiscale microbial diversity. Nature 551, 457–463. https://doi.org/10.1038/nature24621, 7681.
Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M., Lee, Y.K., 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. ISME J. 12 (4), 1072–1083.
Van Der Putten, W.H., 2017. Belowground drivers of plant diversity. Science 355 (6321), 134–135.
Wardle, D.A., 2006. The influence of biotic interactions on soil biodiversity. Ecol. Lett. 9 (7), 870–886.
Weber, P.L., Hermansen, C., Norgaard, T., Pesch, C., Moldrup, P., Greve, M.H., Müller, K., Arthur, E., de Jonge, L.W., 2021. Moisture-dependent water repellency of Greenlandic cultivated soils. Geoderma 402, 115189. https://doi.org/10.1016/j.geoderma.2021.115189.
Whittaker, R.H., 1960. Vegetation of the siskiyou mountains, Oregon and California. Ecol. Monogr. 30 (3), 279–338.
Wu, Y., Zeng, J., Zhu, Q., Zhang, Z., Lin, X., 2017. pH is the primary determinant of the bacterial community structure in agricultural soils impacted by polycyclic aromatic hydrocarbon pollution. Sci. Rep. 7 (1), 1–7.
Xu, Y.X., Du, A., Wang, Z.C., Zhu, W.K., Li, C., Wu, L.C., 2020. Effects of different rotation periods of Eucalyptus plantations on soil T physiochemical properties, enzyme activities, microbial biomass and microbial community structure and diversity. For. Ecol. Manag. 456, 117683.
Zheng, H., Gao, J., Teng, Y., Feng, C., Tian, M., 2015. Temporal Variations in Soil Moisture for Three Typical Vegetation Types in Inner Mongolia, Northern China. PLoS One 10 (3), e0118964. https://doi.org/10.1371/journal.pone.0118964.