RESEARCH ARTICLE

The biodiversity effect of reduced tillage on soil microbiota

Tobias Guldberg Frøslev [ID]**, Ida Broman Nielsen** [ID]**, Susana Silva Santos** [ID]**,
Christopher James Barnes** [ID]**, Hans Henrik Bruun** [ID]**, Rasmus Ejrnæs** [ID]

Abstract The conversion of natural habitats into farmland
has been a leading cause of species loss worldwide. Here,
we investigated to what extent less intensive soil
disturbance can mitigate this loss. Specifically, we
examined whether reduced soil disturbance by tillage in
agricultural fields could contribute to soil microbial
biodiversity by providing a habitat for species that are
limited by conventional tillage. To do so, we studied the
diversity of soil biotas from three agricultural practices
representing conventional tillage, reduced tillage and no
tillage. Study fields were sampled by taking a bulk soil
sample at the centre and edge of each field. The soil
communities were recorded with environmental DNA
metabarcoding using three molecular markers targeting
bacteria, fungi and eukaryotes. While these three markers
represent the vast majority of biotic variation in the soil,
they will inevitably be dominated by the megadiverse
microbiota of bacteria, microfungi and protists. We found a
significant differentiation in community composition
related to the intensity of tillage. Richness was weakly
correlated to tillage, and more influenced by whether the
sample was taken in the center or the edge of the field.
Despite the significant effect of tillage on composition,
comparisons with natural ecosystems revealed that all 30
study fields were much more similar in composition to
other rotational fields than to more natural habitats,
oldfields and leys. Despite a slightly higher similarity to
oldfields and semi-natural grasslands, the contribution of
no-till soil communities to biodiversity conservation is
negligible, and our results indicate that restoration on set
aside land may contribute more to conservation.

INTRODUCTION

We are facing a global biodiversity crisis, driven by human
exploitation of natural resources (Newbold et al. 2015). At
the national level, assessments have confirmed that biodi-
versity is also declining in Denmark, with most protected
habitats and species stuck in unfavorable conservation
status (Ejrnæs et al. 2011; Fredshavn et al. 2019). The
ultimate cause is anthropogenic destruction or deterioration
of habitats for endangered species. Two different approa-
ches to reverse the decline have emerged: land sparing and
land sharing. With land sparing, areas are designated for
biodiversity conservation and protected against human
exploitation. Land sharing, in contrast, seeks to integrate
production and biodiversity using the same areas (Phalan
et al. 2011).

It is typically difficult or impossible to avoid the
destruction of habitats caused by silviculture and agricul-
ture, given that the causes are instrumental to the resource
exploitation, e.g. logging, tilling, drainage and harvesting.
Despite scientific consensus on land sparing as the most
efficient way to turn the biodiversity crisis around, "sus-
tainable" or "nature-friendly" farming and forestry are
perpetuated as important approaches to biodiversity con-
servation (Paoletti et al. 1992). In agriculture specifically,
reducing soil tillage, or completely abandoning it, has
gained traction recently as an approach to sustainable
farming (Derpsch et al. 2010). Many benefits have been
claimed and reported, with emphasis on soil carbon
sequestration (e.g. Chivenge et al. 2007; Zandersen et al.

2016), but positive effects of reduced tillage on the diversity and abundance of soil fauna have also been reported (Brennan et al. 2006). Importantly, no tillage has been shown to also reduce yields compared to reduced tillage and conventional tillage strategies (López-Garrido et al. 2014), and the economic viability of reduced tillage needs to also be compelling for the strategy to be uptaken widely. Metabarcoding of soil DNA has been used in several studies attempting to address the effect of tillage on microbial communities that are otherwise difficult to survey with classical methods. For example, Legrand et al. (2018) assessed the effect of tillage on the diversity of bacteria and fungi, and found effects on community composition and some positive effect of reduced tillage on diversity indices, but not on richness. Taylor et al. (2018) noted that longterm no-till management significantly enhanced the size of soil microbial communities while negatively impacting bacterial diversity. Sengupta and Dick (2015) concluded that higher tillage intensity leads to fewer and more dominant bacterial species.

Although the negative effect of tillage on soil mesofauna can be explained by the destructive impact of mechanical soil perturbation on soil structure and soil fauna (de Graaff et al. 2019), the results of comparative studies are ambiguous. These include immediate (four weeks) positive effects of reduced tillage on earthworm biomass levelling out in spring (Moos et al. 2016), increasing abundance and species richness of collembola (Coulibaly et al. 2017), but also negative effects of reduced tillage on the diversity of soil fungi and bacteria (Degrune et al. 2016). Recent manipulative experiments indicate that the soil biota does not only depend on ecological properties and disturbance regimes, but also on the historical context, i.e. the colonization and extinction of species (Bender et al. 2016). The importance of historical contingency for community assembly has also been shown in secondary succession of vascular plants (e.g. Ejrnæs et al. 2006). Although, several studies have addressed effects of tillage regimes on soil microbial communities and richness, no studies have to our knowledge combined these analyses with a reference dataset to address to what extend the soil communities of the agricultural soils bear resemblance to more natural habitats.

In this study, we compared soil biota composition, richness, biological uniqueness and natural richness between three contrasting soil disturbance regimes: Conventional tillage (CT), reduced tillage (RT) and no-till (NT) conservation agriculture. Assuming a likely biotic interaction with colonization from neighbouring habitats, we sampled soil communities from field edges and from the field centres. We used data from the national biodiversity study Biowide (Brunbjerg et al. 2019) as a reference dataset from which we calculated the biotic uniqueness of all observed species (OTUs) using the *uniquity* score (Ejrnæs et al. 2018) as well as compiling a list of species (OTUs) connected to natural or semi-natural habitats. We studied the genetic diversity of soil microbiota under the assumption that this biota is a good proxy for the composition of the total community of organisms (Ejrnæs et al. 2018; Fløjgaard et al. 2019). Also, the studied species groups are species rich providing representative samples and the place of observation equals the habitat.

Acknowledging the massive diversity, as well as sampling, sorting and identification challenges of traditional methods, we applied environmental DNA metabarcoding to assess the richness and composition of the studied soil communities. Metabarcoding is increasingly used to assess biodiversity of highly complex samples (e.g. soil) and the diversity of hyperdiverse taxa (e.g. bacteria, fungi, protozoa). We extracted DNA from bulk soil samples and subjected this to DNA metabarcoding, using primers targeting bacteria, fungi and general eukaryotes.

Our overall study aim was to assess the potential of reduced soil tillage to contribute to halting biodiversity loss by providing habitat for species that have become locally extinct due to intensive agricultural practices. We approached this objective by investigating the following questions:

- Do different tillage regimes result in significantly different soil biota composition and richness?
- Is the soil biota composition more similar to biotas of natural ecosystems under reduced tillage?
- Can reduced tillage provide habitat for unique biota or biota occurring in natural or semi-natural habitats, but not conventional tillage?

MATERIALS AND METHODS

Study area

We investigated three different soil disturbance regimes, resulting from three different agricultural practices: Conventional tillage (CT), reduced tillage (RT) and no-till (NT) conservation agriculture. The study fields were selected from the acreages of three properties with intermingled fields situated in central Zealand, Denmark. In total, 15 agricultural fields were selected for studying, five from each of the three agricultural practices. The five fields with NT regime (conservation agriculture) have not been tilled since 2000, and from 2008 direct sowing has been the practice. For the RT fields, harrowing-only (no plowing) has been practiced since 2014 (see Electronic Supplementary Material for more details S1). For each field we designated two plots, one representing the central part

(centre), and one representing the edge (edge), resulting in a total of 30 plots (Table S1). The edges represent areas of the field close to neighbouring perennial vegetation and hedges. Edge samples were taken with a distance to the edge of at least two meters from the actual field edge where the field appeared to have a similar appearance to the rest of the field. Each centre plot was approximately 40 × 40 m (allowing for 9 × 9 soil samples in a 4 m spaced grid), and edge plots were adjusted to be longer—aligned with the field perimeter—and allow for 2 × 40/41 or 3 × 27 equally spaced samples. Information on past and present years crops were collected. The fields were sampled in the late autumn (late November to early December) 2018.

Sampling, sequencing and bioinformatics

From each plot we collected one large bulk soil sample consisting of 81 smaller soil cores pooled together and mixed thoroughly. We produced eDNA metabardoding data for bacteria, fungi and eukaryotes for all 30 plots. Protocols for sampling, laboratory work and bioinformatics followed the procedures in Brunbjerg et al. (2019) and Frøslev et al. (2017, 2019). In short, we amplified and sequenced marker genes targeting bacteria, fungi and eukaryotes, and used protocols shown to give sequence data adequate for addressing questions on biodiversity. The results of the bioinformatics are so called OTU tables similar to classical species-site matrices, where OTUs (operational taxonomic units) represent unique sequences, which are used as proxies for species in analyses of biotic richness and composition. Detailed information is given in the Electronic Supplementary Material S1. Although the fungi and eukaryote marker genes also capture multicellular organisms, the three resulting datasets were dominated by soil microorganisms such as bacteria, protists and microfungi. It is an assumption of the study that compositional data from soil mictobiota is a reasonable proxy for terrestrial habitat gradients and compositional turnover of multicellular organisms such as earthworms, beetles, spiders, moths and macrofungi. In order to validate this assumption, we analyzed a large reference data set (Brunbjerg et al. 2019) and reported the correlation between compositional gradients of soil microbiota, environmental gradients and compositional gradients of a multitaxon survey data set (Electronic Supplementary Material S2.2).

Statistical analyses

For each dataset (bacteria, fungi and eukaryotes), we performed the same set of analyses to address potential effects of differences in disturbance regime—CT, RT and NT—

and of plot position (edge or centre). Analyses were carried out in R (v. 3.6.2).

Within-experiment composition and richness

In order to assess whether differential soil disturbance treatment resulted in significantly different microbial richness, we used OTU richness as a proxy for biotic richness. To account for uneven sequencing depth, we used OTU tables resampled to the level of the first quartile using the function *rrarefy* (library *vegan*) in all analyses. OTU richness was calculated as the mean OTU richness from 25 different resampled datasets. To test for experimental effects on the richness of the soil biota we applied linear mixed models with disturbance regime (CT/RT/NT) and position (centre/edge) and their interaction as fixed effects, and crop as random effect. Crop was coded as a factor using the combination of the 2018 crop and the subsequent plant cover at the time of sampling (catch crop or next year's crop), each coded as grass (wheat, rye), legume (horse bean), crucifer (oilseed rape) or none (application of herbicide) for a total of six classes.

Using R library *nlme*, we first tested a mixed-effects model (function *lme*) against a generalized least squares model without random effect (function = *gls*). Secondly, we tested a model including interactions of position and disturbance regime against a simple additive model. Candidate models were compared by function *anova*, using $p < 0.05$ as criterion for accepting the more complex model (likelihood-ratio test). Quantile–quantile plots were used to assess if the data plausibly came from a normal distribution.

We applied multivariate statistics (library *vegan*) to visualize and test for experimental effects on the composition of the soil biota. A Sørensen dissimilarity matrix was produced from the resampled OTU table using the function *vegdist*, and a two-dimensional non-metric multidimensional ordination (NMDS, $k = 2$) was produced with *metaMDS* (default options except for try = 500, trymax = 4000). We tested whether the community dissimilarities showed equal dispersion (beta-dispersion) across all groups (combinations of agricultural practice and position) using the *betadisper* function (library *vegan*). We then tested for effects of location in ordination space using PERMANOVA as implemented in the *adonis2* function using crop type, disturbance regime and in-field position as potential explanatory variables, using marginal testing.

Similarity to natural habitats

To address whether reduced soil disturbance will result in a community composition that is more similar to less

disturbed habitats, such as oldfields and permanent grass-lands, sequence data was combined with the Biowide reference dataset with sequence data from 130 plots across the Danish landscape, representing natural to semi-natural habitats, as well as some agricultural and silvicultural land-use types, representing 26 strata (see Brunbjerg et al. (2019) for more information). For the present study, we compared with 21 pre-defined strata, representing the 18 possible combinations of positions along gradients in soil moisture (dry, moist, wet), successional stage (early, mid, late) and soil fertility (poor, rich), as well as three agri-cultural strata (rotational field, oldfield and ley). Each stratum was represented by 5 replicates ($n = 105$) dis-tributed across Denmark. We resampled the combined OTU table (study + reference), to the level (sequencing depth) of the first quartile, and calculated a Sørensen dis-similarity matrix.

The dissimilarity of each of the 30 study plots to the centroids of each of the 21 strata from the reference dataset was calculated from the dissimilarity matrix using the function *dist_to_centroids* (library *usedist*). Differences in distance to centroids were assessed with Tukey's Honest Test for multiple comparisons ($p < 0.05$). Two NMDS ordinations were calculated for each dataset with the same setting as above, the first including the above mentioned 21 strata of the reference data, and the second restricted to include only the four reference strata most similar to our study sites (which turned out to be rotational fields, oldfields, leys and the MidDryRich stratum for all study treatments).

Treatment effects on uniqueness

Finally, we addressed whether reduced soil disturbance provides habitat for unique biota or biota occurring in natural (or semi-natural) habitats. We defined "natural" OTUs as those OTUs observed among the 18 natural strata of the reference dataset, and calculated the richness of natural OTUs in each of the 30 study plots. Furthermore, we estimated the biotic uniqueness of each plot, using the *uniquity* metric which is a measure of the contribution of a site to the gamma diversity of the surrounding study region. (Ejrnæs et al. 2018). We used the *uniquity* score of each OTU observed in the 18 semi-natural strata of the reference data to calculate the *uniquity* of the 30 study plots by summing uniquity scores of all scored OTUs found also in these plots.

In order to test for experimental effects on the "natural" OTU richness and uniqueness of the soil biota, we applied linear mixed models with disturbance regime (CT/RT/NT) and position (centre/edge) and their interaction as fixed effects, and crop as random effect, in the same way as described under richness analyses above, including

inspection of homoscedasticity and normality of residuals in diagnostic plots.

RESULTS

Soil biota richness and composition

The sequencing resulted in a bacterial dataset of 1 176 623 reads of 19 627 OTUs, a fungal dataset of 3 158 926 reads of 2800 OTUs, and a eukaryote dataset contained 1 936 584 reads of 9725 OTUs. In the bacteria data 0.3% of the OTUs were assigned as Archaea and 0.03% as Eukaryota, but as the propotions were so low and OTUs may well be misassigned bacterial reads we did not exclude them. The eukaryote data was dominated by protistan and amoebo-zoan taxa (28% Cercozoa, 7% Lobosa, 6% Ciliophora, 4% Conosa, etc.), but also a significant proportion of at least partly multicellular taxa (16% Fungi, 8% Metazoa). Some fungal phyla (Cryptomycota, Chytridiomycota) were sig-nificantly better represented in the eukaryote data, and thus we chose to keep all fungal reads as well as unassigned reads in the eukaryote analyses (see Electronic Supple-mentary Material S1 for more information), thereby also retaining the least biased sample of the soil eukaryote community.

Looking exclusively at the project data set (30 plots), bacteria, fungi and eukaryotes all showed negligible dif-ferences in OTU richness caused by tillage regime—except that RT was associated with higher eukaryotic richness. We did however find significant differences between edge and centre position within fields (Table 1, Fig. 1d–f). The field edge had significantly higher OTU richness for fungi and eukaryotes, but (insignificantly) lower richness of bacteria. A random effect of crop types was only included in the model of eukaryote richness.

OTU composition showed a pattern different from richness. The NMDS ordinations (Fig. 1a–c) showed that soil biota depends on soil disturbance regime, with reduced tillage (RT) taking an intermediate position between con-ventional tillage (CT) and no-till (NT). The beta-dispersion test revealed no significant differences in dispersion across combinations of soil disturbance and position (Table 2). Permutation tests for different location in ordination space showed the effect of tillage regime to be significant for all groups of biota. This separation supports the apparent compositional gradient (from CT, through RT to NT) evident for all three primers (Fig. 1a–c). Plot position (edge/centre) had a small but significant location effect for fungi, but no effect for eukaryotes or bacteria (Table 2). Crop had a significant and relatively large effect on the composition of fungi and eukaryotes.

Table 1 Site OTU richness. Table shows model outputs for linear mixed effect modelling of plot ($n = 30$) OTU richness of bacteria, fungi and eukaryotes, and with tillage regime and plot position (edge/centre) as explanatory variables. Estimates and p values (*< 0.05, ** < 0.01, ***< 0.001) are given. No significant interactions were detected and are left out of the table. Crop (random) indicates whether the model was improved by adding the crop variable as random effect, which was only the case for eukaryotes. The only significant effects on richness with increased tillage (compare to conventional tillage) is for reduced tillage for eukaryotes. Plot position at field edge contributed to richness for fungi and eukaryotes

Richness	Bacteria	Fungi	Eukaryotes
Intercept	1595.1***	273.3***	527.8***
Tillage, disturbance RT	36.7	− 11.3	243.1**
Tillage, disturbance NT	20.6	− 1.4	64.4
Position, edge	− 95.3	54.8***	122.6*
Crop (random effect)	No	No	Yes*

Table 2 Compositional differences. Table shows results from PERMANOVA tests for compositional differences assigned to tillage regimes, position in the field and crop types for bacteria, fungi and eukaryotes. R-squared values and p values (*< 0.05, ** < 0.01, *** < 0.001) are given. Betadispersion indicates whether significant differences in multivariate heterogeneity/homogeneity of sample dispersion was detected between groups. Tillage regime had a significant effect of composition for all three organism groups, and position for fungi, and crop for fungi and bacteria were also significant. There was no significant differences in the level of dispersion for the three organism groups

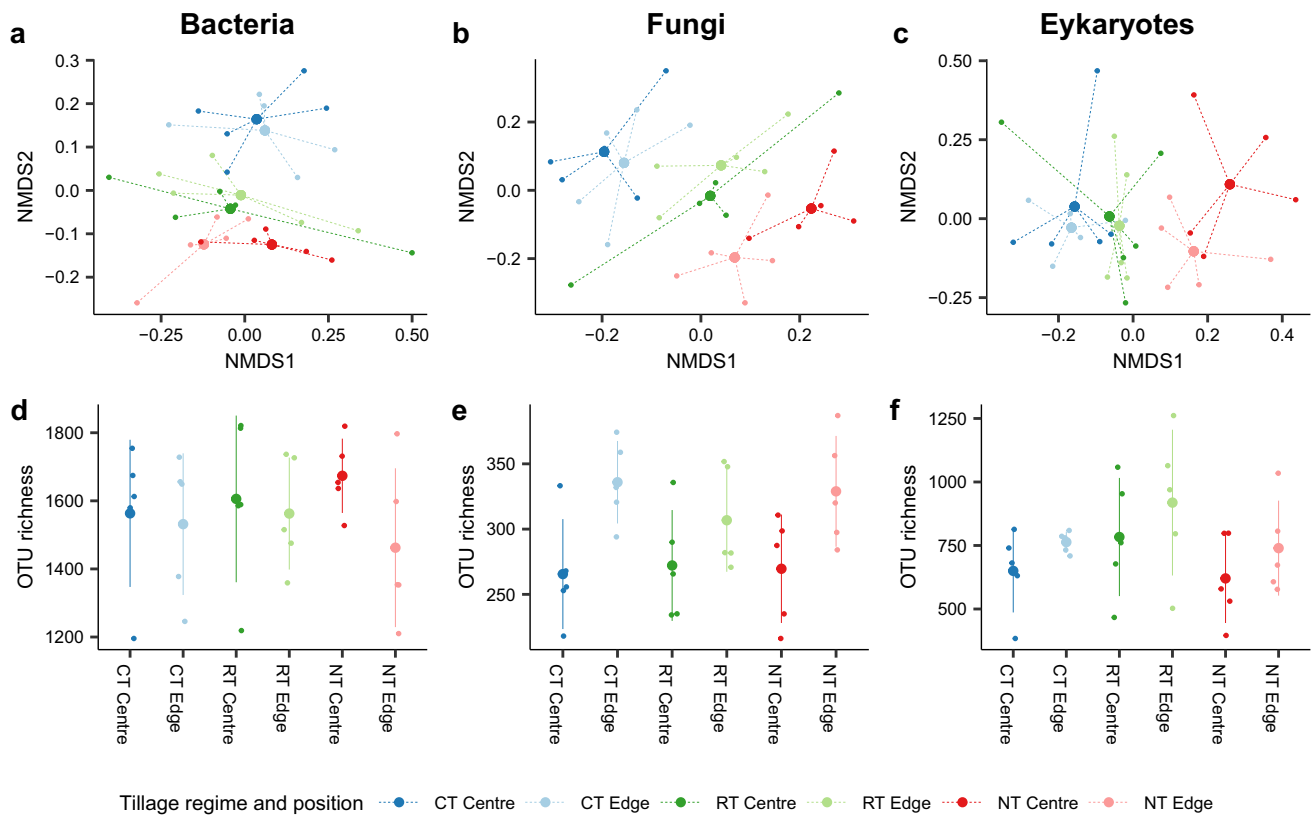| Permanova	Bacteria	Fungi	Eukaryotes
Tillage regime	0.10**	0.11***	0.09***
Position in field	0.03	0.04*	0.03
Crop	0.18	0.20***	0.19***
Betadispersion	No	No	No

Fig. 1 Composition and richness of OTUs. Plots show sampling plots coloured according to regime (blue = CT, green = RT and red = NT) and shaded according to position (dark = centre, pale = edge). **a–c** NMDS ordination of Sørensen dissimilarity of the three organism groups, based on OTU tables resampled to even sequencing depth. Euclidean centroids (means along NMDS axes) are marked for each class. **d–f** OTU richness of the six classes. y-axis indicates the OTU richness of plots. For each class the mean ± 2*sd is indicated

Similarity to natural habitats

Data from the current study (30 plots) was compared against a larger reference data set, which spans the overall variation in terrestrial habitats in Denmark (Brunbjerg et al. 2019). It was revealed that the fields from this study were more similar to other rotational fields than to any other terrestrial habitat type and furthermore that the fields were more similar to oldfields and improved grass leys than to natural habitats such as dry grassland, meadow, mire, heathland and woodland (Fig. 2). This pattern was evident for all biotic groups (bacteria, fungi and eukaryotes), soil treatments (conventional, reduced and no tillage) and plot position (centre/edge). The natural habitat type most similar to our study fields was dry and neutral-calcareous grassland with closed vegetation cover (MidDryRich stratum, highlighted in black in Fig. 2).

Narrowing the comparison to only include rotational fields, oldfields, leys and dry semi-natural grassland (MidDryRich), the biotas of field edges were generally found to be more similar to natural habitats than biotas of field centres (Fig. 2). Further, no-till (NT) was slightly more similar to grassland, oldfield and ley and less similar to rotational fields than were reduced tillage (RT) and conventional tillage (CT) (Fig. 2b and c). The figures show the same overall trends with decreasing similarity (increasing distance to reference centroid) of study fields to rotational fields in the reference data with lower levels of soil disturbance, and conversely increasing similarity (decreasing distance to reference centroid) of study fields to grassland, oldfield and ley with lower levels of soil disturbance. Despite these tendencies, only some differences were significant as seen from the letters indicating groups of post-hoc Tukey HSD tests in Fig. 2.

Biotic uniqueness and OTUs shared with natural habitats

Field edge plots had significantly higher numbers of fungal OTUs in common with natural habitats than the field centres (Table 3, Fig. 3). No difference in response to tillage regime was found, however. For bacterial and eukaryotic OTUs, no significant differences were observed, but bacteria revealed the opposite pattern of fungi and eukaryotes, with the highest richness of natural habitat species in the field centres.

We found a significantly higher *uniquity* score for fungi in the field edges, and for eukaryotes in fields with reduced tillage (RT) (Table 4). As for the natural richness, bacteria revealed the opposite (but insignificant) pattern of fungi and eukaryotes, with the highest uniquity in the field centres.

DISCUSSION

The biodiversity crisis hits broadly—particularly through the destruction of habitats. Habitat quality and biotic community intactness is assumed to be well represented by the richness and composition of soil communities responding to changes in moisture, nutrients, carbon substrates and temporal continuity. No attempt has yet been made to assess the conservation status or 'threatenedness' of soil microbiota, but we find it likely that also less known communities of fungi, mites, collembola and protozoa may include organisms that are constrained by habitat destruction.

Land sharing has the basic idea of accommodating biodiversity, including all the commensal or neutral species, not just those beneficial to crop production. If higher levels of biodiversity could be accommodated in certain cultivation systems, without being at the expense of yield, or some similar expression of the farmer's interest, then it takes no further motivation. It would constitute evidence in favor of land sharing. Reduced tillage has been proposed as a measure to promote higher levels of soil biodiversity, while retaining agricultural production, and thus an implementation of land sharing in practice.

Our investigation of soil biodiversity effects of reduced tillage and no-till used eDNA metabarcoding to target the three most species-rich components of the soil microbiota (bacteria, fungi and eukaryotes). Overall, we found small differences in OTU richness, but a significant differentiation in the OTU composition related to the intensity of tillage. No-till agriculture promotes soil biodiversity with higher similarity to oldfields and slightly higher to semi-natural grasslands, but very far from the full suite of natural habitats.

Richness and composition

We found a significant differentiation in the composition of the soil biota related to soil tillage intensity as a clear gradient from conventional tillage over reduced tillage to no till. Richness patterns were weakly related to tillage, but more influenced by proximity to field surroundings, i.e. whether samples were taken in the centre or the edge of a field. Comparing the study fields with reference data from more or less natural ecosystems, revealed that—despite significant effects of cultivation methods on soil biota composition—all 30 study fields were much more similar in composition with reference rotational fields than they were to more natural habitats or oldfields and leys.

Our results are seemingly at odds with those of Tsiafouli et al. (2015), who convincingly showed how increased land-use intensity leads to reduced species richness of earthworms, springtails and oribatid mites in agricultural
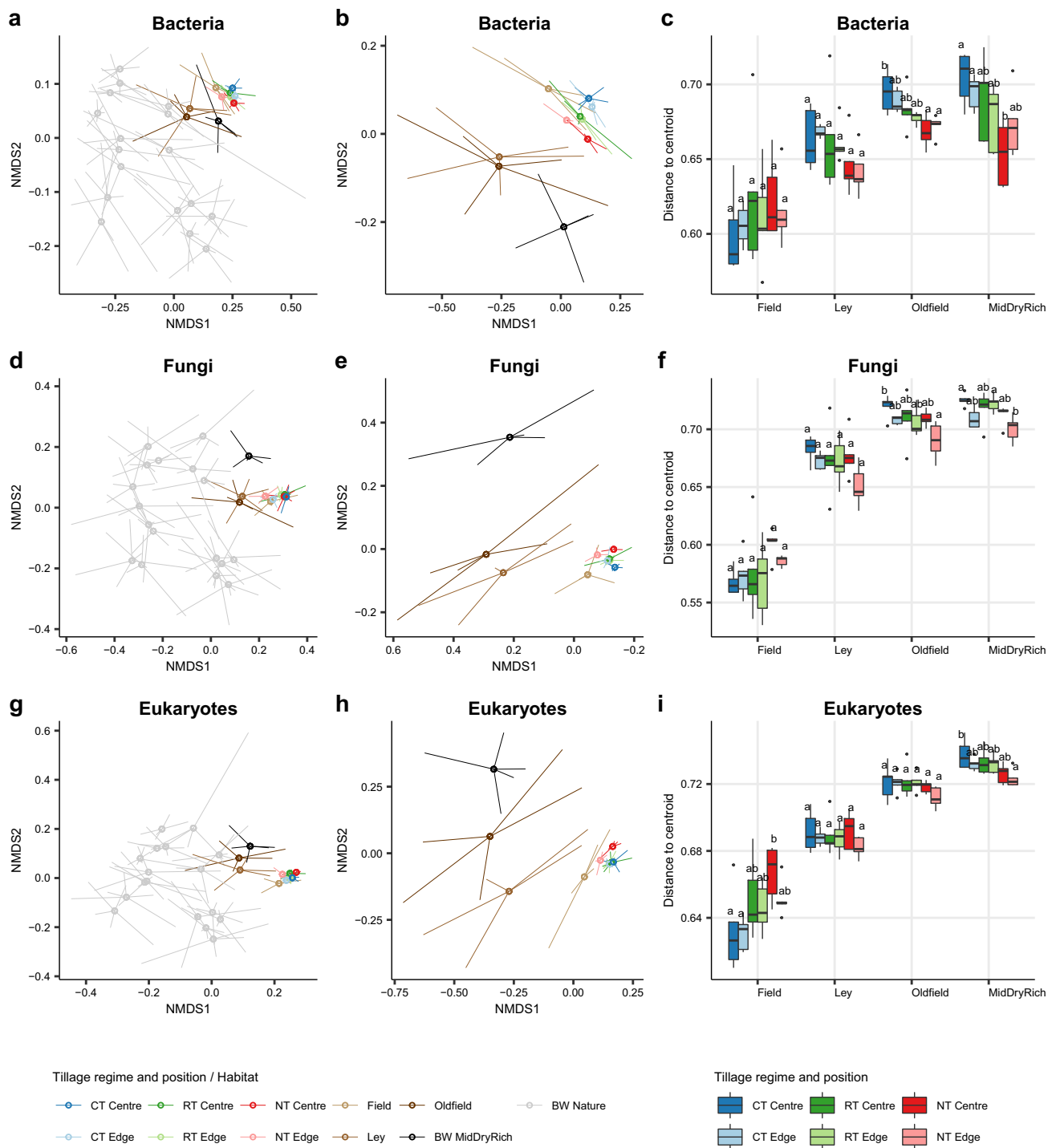
Fig. 2 Similarity to natural habitats. Charts show study plots coloured according to disturbance regime (blue = CT, green = RT and red = NT) and shaded according to position (dark = centre, pale = edge), reference data are colored according to designated stratum (light brown = conventional rotational field, brown = ley grass field, dark brown = oldfield, black = MidDryRich stratum (dry and neutral-calcareous grassland with "closed" vegetation cover), light grey = remaining 17 seminatural strata). Upper row shows data for bacteria, middle for fungi and lower for eukaryotes. Left column (**a, d, g**): NMDS ordination of the three organism groups of the study plots (*n* = 30) as well as the reference data (*n* = 105) representing different agricultural and semi-natural habitats. Euclidean centroids are marked for each class. Middle column (**b, e, h**): Similar, but restricted to the four most relevant habitat types of the reference data, i.e. three agricultural strata and permanent dry circumneutral grassland (*n* = 25). Right column (**c, f, i**): Distance to centroids of the four most relevant habitat types of the reference data from study plots in each of the six treatments in the study data. *X*-axis indicates the target centroid of the four reference data centroids (field, ley, oldfield and dry grassland). *Y*-axis (truncated) indicates the distance of the study plots to the centroid. For each target centroid separately, letters indicate significant differences between treatments (defined by disturbance and position) by post-hoc Tukey HSD tests

Table 3 Natural OTU richness. Table shows model output for linear mixed effect modelling of plot (n = 30) richness of 'natural' OTUs of bacteria, fungi and eukaryotes, with tillage regime and plot position (edge/centre) as explanatory variables. Estimates and p values (*< 0.05, ** < 0.01, *** < 0.001) are given. No significant interactions were detected and are left out of the table. Crop (random) indicates whether the model was improved by adding the crop variable as random effect, which was not the case for any of the groups. There were no significant effects on richness with increased tillage (compare to conventional tillage). Plot position at field edge had a significant effect on richness for. Natural OTUs are defined as those observed among 18 natural strata of the reference dataset of soils from natural and seminatural habitats

Richness of 'natural' OTUs	Bacteria	Fungi	Eukaryotes
Intercept	1005.9***	191.9***	409.8***
Tillage, RT	38.1	− 8.3	76.7
Tillage, NT	75.5	− 1.7	− 6.8
Position, edge	− 48.7	37.5**	72.9
Crop (random)	No	No	No

Table 4 Biotic uniqueness. Table shows model output for linear mixed effect modelling of plot (n = 30) uniquity of bacteria, fungi and eukaryotes, with tillage regime and plot position (edge/centre) as explanatory variables. Estimates and p values (*< 0.05, ** < 0.01, *** < 0.001) are given. No significant interactions were detected and are left out of the table. Crop (random) indicates whether the model was improved by adding the crop variable as random effect, which was only the case for fungi. The only significant effects on richness with tillage (compare to conventional tillage) is for reduced tillage for eukaryotes. Plot position at field edge contributed to richness for fungi. Biotic uniqueness was calculated with the *uniquity* metric which is a measure of the contribution of a site to the gamma diversity of the surrounding study region (Ejrnæs et al. 2018)

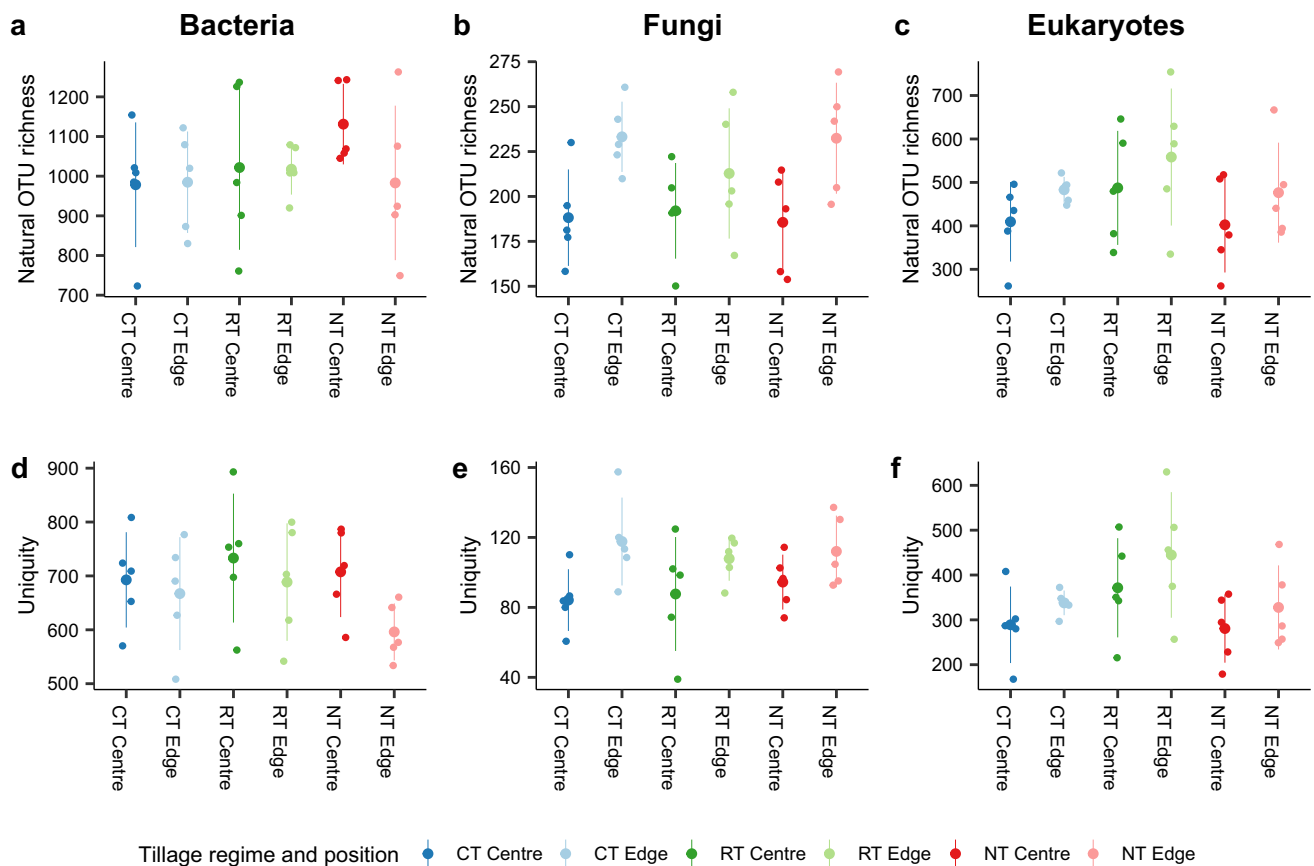| Uniquity	Bacteria	Fungi	Eukaryotes
Intercept	710.2***	77.1***	285.2***
Tillage, RT	30.9	8.1	94.7*
Tillage, NT	− 28.1	10.4	− 9.2
Position, edge	− 95.7	23.8***	56.4
Crop (random)	No	Yes*	No

Fig. 3 Richness of natural OTUs and biotic uniqueness. Plots show sampling plots coloured according to regime (blue = CT, green = RT and red = NT) and shaded according to position (dark = centre, pale = edge). For each class the mean ± 2*sd is indicated. **a–c** Richness of OTUs defined as natural OTUs (OTUs present in at least one of the plots from natural strata of the reference dataset). **d–f** Uniquity of the 30 study plots. Uniquity scores for each OTU is calculated for the full reference dataset (n = 130), and the *uniquity* of the 30 study plots is calculated as the sum of uniquity scores of OTUs observed in the sample. Left column shows bacteria, middle column fungi, and right column eukaryotes. The *uniquity* metric is a measure of the contribution of a site to the gamma diversity of the surrounding study region (Ejrnæs et al. 2018)

soils. However, the study by Tsiafouli et al. (2015) compared rotational fields with long-term crops and permanent grasslands, implying soil maturation and community succession, rather than a gradient in soil perturbation intensity per se. Also, our study is focused primarily on microbial communities, and although our eukaryotic marker targets eukaryotes widely, any signal from metazoans will be masked by the highly dominating groups of protists and fungi.

An early study of the response of soil communities of collembola to tillage, using classical methods for sampling and identification found that reduced or no tillage had no effects on richness, but strong effects on total abundance (Brennan et al. 2006). In contrast, Coulibaly et al. (2017) found reduced tillage promoted both species richness and abundance of collembola. In the same vein, other studies have found richness of surface-dwelling predators and earthworms to increase with reduced tillage (Tsiafouli et al. 2015; Briones and Schmidt 2017; D'Hose et al. 2018). The eukaryotic marker applied in the present study includes soil mesofauna, but still is likely to result in richness and compositional metrics dominated by organisms of much smaller size, which—like soil fungi and bacteria—suffer less mortality and recover much faster following soil perturbation (Jurburg et al. 2017; Meisner et al. 2017). The broad scope of the present study on megadiverse groups could explain the apparent discrepancy.

Tillage practices can effectively shape soil bacteria and fungal communities in terms of abundance and richness (Le Guillou et al. 2019). One may hypothesize that soil disturbance by tillage could counteract the establishment and expansion of dominant species which eventually could result in a higher species richness at intermediate disturbance levels (Grime 1974; Huston 1979). There is, however, much less evidence for this mechanism in soil biotas than for plant communities. Our study does not suggest a consistent response in the OTU richness of fungi, bacteria or eukaryotes to the studied gradient in physical disturbance. We found higher richness of fungi and eukaryotes near the field edges neighbouring perennial vegetation in field margins, road verges and hedges. This is likely a direct effect of the presence of live and dead roots of a variety of trees, shrubs and perennial herbs not living in the proper field and, thus, a special case of ecospace expansion (sensu Brunbjerg et al. 2020). In addition, spill-over of transient species having source populations in neighbouring habitats (Taylor et al. 2018) is likely to contribute as well.

We found a significant difference in soil biota composition, a result underpinning that soil disturbance has importance for the assembly and possibly also the functioning of soil communities. We also found a significant effect of crop combinations on the composition of fungi and eukaryotes, a result corroborating the importance of vascular plants as ecospace for communities of symbionts and decomposers (Brunbjerg et al. 2018).

It has been argued that richness is an unreliable metric in metabarcoding studies due to inherent limitations and biases in the laboratory procedures. Biases certainly exist for very species-rich samples, in which sequencing depth limits the number of detected OTUs and, consequently, observed richness becoming a function of community evenness (Bálint et al. 2016). In contrast to this notion, fungal richness measures derived from eDNA metabarcoding have proved to correlate well with macrofungal richness assessed by classical inventories (Frøslev et al. 2019).

Successional convergence

Reference data enabled us to put our results for agricultural soil biotas into context and assess if soil communities under reduced or ceased tillage are more similar to more perennial biotas than to biotas of rotational fields. We found that the most similar biotas were found in reference rotational fields (organic and conventional alike), irrespective of the intensity of soil disturbance in the study plots. Oldfields and permanent grass leys also showed some similarity to the studied soil communities, but much less than rotational fields. The most similar natural habitat was dry neutral-calcareous grassland. According to the present study, reduced tillage within the time frame of the present study (18 years with no-till and 4–6 years of reduced tillage) is not sufficient to develop arable soil biotas similar to more permanent vegetation of less intensive land use (Tsiafouli et al. 2015).

Contribution to natural or unique biotas

The number of OTUs assigned as natural or unique on a national scale responded similarly to the broader species richness, albeit less significantly so. In the context of the other study results we interpret this as an indication that reduced tillage does not lead to increased occurrence of natural or unique species, but rather impact species that are common to agricultural fields with annual crops. In this respect, our study supports the notion that less intensive agricultural practices may have the strongest effect at very local scale and in intensively cultivated landscapes (Bengtsson et al. 2005; Tuck et al. 2014).

Reduced tillage and biological conservation

It has been argued from a theoretical point of view that extinction risks of soil biota should be paid attention to in conservation ecology (Veresoglou et al. 2015)—not least

based on the notion that the composition of soil biotas have important effects on ecosystem functioning (Brussaard et al. 2007). In this study, we used a large reference data set to assess the uniqueness and naturalness of agricultural soils under conventional and reduced tillage farming. Although we demonstrated significant changes in community composition in response to reduced tillage and no-till, we did not find significant changes in species richness, uniqueness or naturalness. In principle, we cannot preclude that the absence of conservation value associated with conservation agriculture is caused by dispersal limitation causing a delayed recovery time. The positive edge effect on uniqueness and naturalness of fungi and eukaryotes could point in this direction, but the effect was insignificant and even edge samples remained much more similar to rotational fields than to soil biotas of more permanent terrestrial habitats. In accordance with recent studies of terrestrial multi-taxon diversity (Brunbjerg et al. 2020) we find it more likely that even in the absence of tillage, the biota of agricultural soils is strongly filtered to fit a uniform abiotic environment and homogenous supply of organic substrates dominated by a low-diverse rotation of annual crops. While reduced tillage leads to rapid changes in soil physical properties (Kuhwald et al. 2017), Turley et al. (2020) and Strickland et al. (2017) observed that the legacies of agricultural land use are difficult for the soil biota to overcome. Post-agricultural communities seemingly do not recover to similar remnant communities, with changes happening very slowly or being contingent on restoration of plant community composition.

While the observed compositional differentiation of soil biotas may imply differences in ecosystem functioning with implications for crop production, our study did not test this hypothesis, and we are reluctant to place too much emphasis on the relatively minor compositional differences observed. When it comes to the wider conservation of natural soil biotas threatened by historical land-use intensification we found no evidence for an important contribution of reduced tillage. Thus, our results contradict the feasibility of land sharing as an approach to conserve and restore regional soil biodiversity. Instead, our results suggest that conservation of regional soil biodiversity is best achieved through a land sparing approach, implying the designation of land set aside for conservation of natural forest, grassland, heathland and wetland ecosystems.

REFERENCES

Bálint, M., M. Bahram, A.M. Eren, K. Faust, J.A. Fuhrman, B. Lindahl, R.B. O'Hara, M. Öpik, et al. 2016. Millions of reads, thousands of taxa: Microbial community structure and associations analyzed via marker genes. *FEMS Microbiology Reviews* 40: 686–700.

Bender, S.F., C. Wagg, and M.G. van der Heijden. 2016. An underground revolution: Biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology & Evolution* 31: 440–452.

Bengtsson, J., J. Ahnström, and A.C. Weibull. 2005. The effects of organic agriculture on biodiversity and abundance: A meta-analysis. *Journal of Applied Ecology* 42: 261–269.

Brennan, A., T. Fortune, and T. Bolger. 2006. Collembola abundances and assemblage structures in conventionally tilled and conservation tillage arable systems. *Pedobiologia* 50: 135–145.

Briones, M.J.I., and O. Schmidt. 2017. Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. *Global Change Biology* 23: 4396–4419.

Brunbjerg, A.K., H.H. Bruun, K. Brøndum, A.T. Classen, L. Dalby, K. Fog, T.G. Frøslev, I. Goldberg, et al. 2019. A systematic survey of regional multi-taxon biodiversity: Evaluating strategies and coverage. *BMC Ecology* 19: 1–15.

Brunbjerg, A.K., H.H. Bruun, L. Dalby, A.T. Classen, C. Fløjgaard, T.G. Frøslev, O.L.P. Hansen, T.T. Høye, et al. 2020. Multi-taxon inventory reveals highly consistent biodiversity responses to ecospace variation. *Oikos* 129: 1381–1392.

Brunbjerg, A.K., H.H. Bruun, L. Dalby, C. Fløjgaard, T.G. Frøslev, T.T. Høye, I. Goldberg, T. Læssøe, et al. 2018. Vascular plant species richness and bioindication predict multi-taxon species richness. *Methods in Ecology and Evolution* 9: 2372–2382.

Brussaard, L., P.C. De Ruiter, and G.G. Brown. 2007. Soil biodiversity for agricultural sustainability. *Agriculture, Ecosystems & Environment* 121: 233–244.

Chivenge, P.P., H.K. Murwira, K.E. Giller, P. Mapfumo, and J. Six. 2007. Long-term impact of reduced tillage and residue management on soil carbon stabilization: Implications for conservation agriculture on contrasting soils. *Soil and Tillage Research* 94: 328–337.

Coulibaly, S.F., V. Coudrain, M. Hedde, N. Brunet, B. Mary, S. Recous, and M. Chauvat. 2017. Effect of different crop management practices on soil Collembola assemblages: A 4-year follow-up. *Applied Soil Ecology* 119: 354–366.

de Graaff, M.-A., N. Hornslein, H.L. Throop, P. Kardol, and L.T. van Diepen. 2019. Effects of agricultural intensification on soil biodiversity and implications for ecosystem functioning: A meta-analysis. *Advances in Agronomy* 155: 1–44.

Degrune, F., N. Theodorakopoulos, M. Dufrêne, G. Colinet, B. Bodson, M.P. Hiel, B. Taminiau, C. Nezer, et al. 2016. No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium). *Agriculture, Ecosystems & Environment* 224: 12–21.

Derpsch, R., T. Friedrich, A. Kassam, and H. Li. 2010. Current status of adoption of no-till farming in the world and some of its main

benefits. *International Journal of Agricultural and Biological Engineering* 3: 1–25.

D'Hose, T., L. Molendijk, L. Van Vooren, W. van den Berg, H. Hoek, W. Runia, F. van Evert, H. ten Berg, et al. 2018. Responses of soil biota to non-inversion tillage and organic amendments: An analysis on European multiyear field experiments. *Pedobiologia* 66: 18–28.

Ejrnæs, R., H.H. Bruun, and B.J. Graae. 2006. Community assembly in experimental grasslands: Suitable environment or timely arrival? *Ecology* 87: 1225–1233.

Ejrnæs, R., T.G. Frøslev, T.T. Høye, R. Kjøller, A. Oddershede, A.K. Brunbjerg, A.J. Hansen, and H.H. Bruun. 2018. Uniquity: A general metric for biotic uniqueness of sites. *Biological Conservation* 225: 98–105.

Ejrnæs, R., P. Wiberg-Larsen, T.E. Holm, A. Josefson, B. Strandberg, B. Nygaard, L.W. Andersen, A. Winding, et al. 2011. Danmarks biodiversitet 2010—Status, udvikling og trusler. Faglig rapport fra DMU nr. 815. Danmarks Miljøundersøgelser, Aarhus Universitet. Aarhus, Denmark (in Danish) (Report)

Fløjgaard, C., T.G. Frøslev, A.K. Brunbjerg, H.H. Bruun, J. Moeslund, A.J. Hansen, and R. Ejrnæs. 2019. Predicting provenance of forensic soil samples: Linking soil to ecological habitats by metabarcoding and supervised classification. *PLoS ONE* 14: e0202844.

Fredshavn, J.R., B. Nygaard, R. Ejrnæs, C. Damgaard, O.R. Therkildsen, M. Elmeros, P. Wind, L.S. Johansson, et al. 2019. Bevaringsstatus for naturtyper og arter—2019: Habitatdirektivets Artikel 17-rapportering. Aarhus, Denmark (in Danish) (Report)

Frøslev, T.G., R. Kjøller, H.H. Bruun, R. Ejrnæs, A.K. Brunbjerg, C. Pietroni, and A.J. Hansen. 2017. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications* 8: 1–11.

Frøslev, T.G., R. Kjøller, H.H. Bruun, R. Ejrnæs, A.J. Hansen, T. Læssøe, and J. Heilmann-Clausen. 2019. Man against machine: Do fungal fruitbodies and eDNA give similar biodiversity assessments across broad environmental gradients? *Biological Conservation* 233: 201–212.

Grime, J.P. 1974. Vegetation classification by reference to strategies. *Nature* 250: 26–31.

Huston, M. 1979. A general hypothesis of species diversity. *The American Naturalist* 113: 81–101.

Jurburg, S.D., I. Nunes, A. Brejnrod, S. Jacquiod, A. Priemé, S.J. Sørensen, J.D. Van Elsas, and J.F. Salles. 2017. Legacy effects on the recovery of soil bacterial communities from extreme temperature perturbation. *Frontiers in Microbiology* 8: 1832.

Kuhwald, M., M. Blaschek, J. Brunotte, and R. Duttmann. 2017. Comparing soil physical properties from continuous conventional tillage with long-term reduced tillage affected by one-time inversion. *Soil Use and Management* 33: 611–619.

Le Guillou, C., N. Chemidlin Prévost-Bouré, B. Karimi, N. Akkal-Corfini, S. Dequiedt, V. Nowak, S. Terrat, S. Menasseri-Aubry, et al. 2019. Tillage intensity and pasture in rotation effectively shape soil microbial communities at a landscape scale. *MicrobiologyOpen* 8: e00676.

Legrand, F., A. Picot, J. F. Cobo-Díaz, M. Carof, W. Chen, and G. Le Floch. 2018. Effect of tillage and static abiotics oil properties on microbial diversity. *Applied Soil Ecology* 132: 135–145.

López-Garrido, R., E. Madejón, M. León-Camacho, I. Girón, F. Moreno, and J.M. Murillo. 2014. Reduced tillage as an alternative to no-tillage under Mediterranean conditions: A case study. *Soil and Tillage Research* 140: 40–47.

Meisner, A., A. Leizeaga, J. Rousk, and E. Bååth. 2017. Partial drying accelerates bacterial growth recovery to rewetting. *Soil Biology and Biochemistry* 112: 269–276.

Moos, J.H., S. Schrader, H.M. Paulsen, and G. Rahmann. 2016. Occasional reduced tillage in organic farming can promote earthworm performance and resource efficiency. *Applied Soil Ecology* 103: 22–30.

Newbold, T., L.N. Hudson, S.L.L. Hill, S. Contu, I. Lysenko, R.A. Senior, L. Börger, D.J. Bennet, et al. 2015. Global effects of land use on local terrestrial biodiversity. *Nature* 520: 45–50.

Paoletti, M.G., D. Pimentel, B.R. Stinner, and D. Stinner. 1992. Agroecosystem biodiversity: Matching production and conservation biology. *Agriculture, Ecosystems & Environment* 40: 3–23.

Phalan, B., M. Onial, A. Balmford, and R.E. Green. 2011. Reconciling food production and biodiversity conservation: Land sharing and land sparing compared. *Science* 333: 1289–1291.

Sengupta, A., and W.A. Dick. 2015. Bacterial community diversity in soil under two tillage practices as determined by pyrosequencing. *Microbial Ecology* 70: 853–859.

Strickland, M.S., M.A. Callaham Jr., E.S. Gardiner, J.A. Stanturf, J.W. Leff, N. Fierer, and M.A. Bradford. 2017. Response of soil microbial community composition and function to a bottomland forest restoration intensity gradient. *Applied Soil Ecology* 119: 317–326.

Taylor, S.J., B.S. Evans, E.P. White, and A.H. Hurlbert. 2018. The prevalence and impact of transient species in ecological communities. *Ecology* 99: 1825–1835.

Tsiafouli, M.A., E. Thébault, S.P. Sgardelis, P.C. De Ruiter, W.H. Van Der Putten, K. Birkhofer, L. Hemerik, F.T. de Vries, et al. 2015. Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biology* 21: 973–985.

Tuck, S.L., C. Winqvist, F. Mota, J. Ahnström, L.A. Turnbull, and J. Bengtsson. 2014. Land-use intensity and the effects of organic farming on biodiversity: A hierarchical meta-analysis. *Journal of Applied Ecology* 51: 746–755.

Turley, N.E., L. Bell-Dereske, S.E. Evans, and L.A. Brudvig. 2020. Agricultural land-use history and restoration impact soil microbial biodiversity. *Journal of Applied Ecology* 57 (5): 852–863.

Veresoglou, S.D., J.M. Halley, and M.C. Rillig. 2015. Extinction risk of soil biota. *Nature Communications* 6: 1–10.

Zandersen, M., S.L. Jørgensen, D. Nainggolan, S. Gyldenkærne, A. Winding, M.H. Greve, and M. Termansen. 2016. Potential and economic efficiency of using reduced tillage to mitigate climate effects in Danish agriculture. *Ecological Economics* 123: 14–22.

AUTHOR BIOGRAPHIES

Tobias Guldberg Frøslev (✉) is an Associate Professor, at the GLOBE Institute, University of Copenhagen. His research interests include biodiversity, eDNA metabarcoding, fungal diversity and ecology.
Address: Section for Geogenetics, GLOBE Institute, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen, Denmark.
e-mail: tobiasgf@sund.ku.dk

Ida Broman Nielsen is a Research Assistant, at the GLOBE Institute, University of Copenhagen. Her research interests include eDNA metabarcoding and sequence analysis.
Address: Section for Geogenetics, GLOBE Institute, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen, Denmark.
e-mail: ida.broman.nielsen@sund.ku.dk

Susana Silva Santos is a Post Doc at the Department of Agroecology, Aarhus University. Her research interests include protozoology, microbial biodiversity and ecology, and eDNA metabarcoding.
Address: Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark.
Address: Department of Agroecology, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark.
e-mail: suss@agro.au.dk

Christopher James Barnes is as Assistant Professor, at the GLOBE Institute, University of Copenhagen. His research interests include soil ecology, biodiversity and eDNA metabarcoding.
Address: Section for Geogenetics, GLOBE Institute, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen, Denmark.
e-mail: c.barnes@sund.ku.dk

Hans Henrik Bruun is an Associate Professor, at the Biological Department, University of Copenhagen. His research interests include community ecology, biodiversity, conservation science and human impacts on nature.
Address: Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark.
e-mail: hhbruun@bio.ku.dk

Rasmus Ejrnæs is a Senior Scientist, at the Department of Bioscience, Aarhus University. His research interests include community ecology, nature conservation, restoration and rewilding.
Address: Section for Biodiversity & Conservation, Department of Bioscience, Aarhus University, 8410 Rønde, Denmark.
e-mail: rasmus@bios.au.dk