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Soil heterogeneity and plant species diversity in experimental grassland communities: contrasting effects of soil nutrients and pH at different spatial scales

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Abstract

Background and aims Current knowledge of soil heterogeneity-diversity relationships (HDR) is largely based on studies manipulating single factor, but the advancements in HDR may require a comprehensive experiment incorporating multiple factors.

Methods We conducted a three-year field experiment in which a seed mixture of 16 common grassland species was sown in plots with heterogeneous soils consisting of small (10 cm × 10 cm) or large patches (30 cm × 30 cm) of low and high nutrients or low and high pH, and homogeneous soils with an even mixture of low and high nutrient/pH soils. Soil nutrients and pH were manipulated in separate treatments. We determined plant species richness and diversity at two focal scales (40 cm × 40 cm plot-scale and 10 cm × 10 cm patch-scale).

Results Plot-scale richness and diversity were not influenced by soil heterogeneity, but patch-scale richness was lower in plots with heterogeneous nutrients than in plots where nutrients were distributed homogeneously. There was no difference between the two heterogeneous nutrient soils with different grain sizes. Patch-scale diversity was higher in heterogeneous pH soils of large patch size than in heterogeneous pH soils of small patch size or the homogeneous pH soil at the final harvest. Species richness and diversity quantified at both plot and patch scales declined in all soils over time.

Conclusions The influence of soil heterogeneity on plant species diversity depends on whether the soil varies in nutrients or pH, and on the temporal-spatial scale at which species diversity and soil heterogeneity are measured. These results indicate that soil heterogeneity has the potential to promote plant coexistence and future HDR studies should consider multiple soil factors at various temporal-spatial scales.

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Introduction

Soil heterogeneity is widely thought to promote plant species coexistence and plant species diversity by increasing niche availability (Levine and HilleRisLambers 2009) and by creating shelters and refuges (e.g. competition-free patches) from harsh environmental conditions (e.g. competition-dominant patches; Chesson 2000; Hutchings et al. 2003). This phenomenon, howerver, depends on the spatial scale at which herterogeneity varies (Eilts et al. 2011; Hutchings et al. 2003; Tamme et al. 2010). The environmental heterogeneity hypothesis has been well-supported in theory (Chesson 2000; Hutchings et al. 2003; Ricklefs 1977; Tilman and Pacala 1993) and in numerous observational studies (reviewed in Lundholm 2009; Stein et al. 2014). Only a few experiments, however, have directly tested the effects of small-scale soil heterogeneity on plant species diversity. The few experimental studies have reported mixed results, varying from positive to negative (e.g. Eilts et al. 2011; Gazol et al. 2013; Reynolds et al. 2007; Wijesinghe et al. 2005; Williams and Houseman 2013), but overall, non-positive or weak effects of soil heterogeneity prevail (Lundholm 2009; Tamme et al. 2010).

When the scale of soil heterogeneity (i.e. patch size or grain size) is smaller than the size of the plant rooting system, especially clonal plants, species can exploit favoured patches through selective replacements of ramets or roots, thus outcompeting other plant species (e.g. Day et al. 2003; Fransen et al. 2001; Hutchings and de Kroon 1994). Therefore, when certain species perform better in heterogeneous soils, plant species diversity may decrease (i.e., the environment filter effect; Bazzaz 1991; Kraft et al. 2015; Tamme et al. 2016). Further, Hutchings et al. (2003) predicted that when the scale of soil heterogeneity is larger than the size of the plant rooting system, different soil patches will support distinct sub-communities, and the overall diversity will be higher than in equivalent homogeneous soils.

Soil heterogeneity effects on plant species diversity also depend on the focal scale: the spatial scale at which plant species diversity is quantified. At greater focal scales, the number of microhabitats included in one sample may increase, and may capture greater species coexistence (Allouche et al. 2012; MacArthur and Wilson 1967). However, a meta-analysis on the few experimental studies examining soil heterogeneity effects on plant species diversity showed that the shape and magnitude of heterogeneity-diversity relationships were not related to the focal scale (Lundholm 2009). Therefore, we may expect that the effects of soil heterogeneity on plant species diversity are independent of the focal scale.

Schoolmaster Jr. (2013) proposed that effects of soil heterogeneity on plant species diversity may also depend on whether the soil varies in resource (e.g. soil nutrient or water availability) or non-resource factors (e.g. soil pH and soil type) because non-resource factors have important impacts on the competitive vigour of plants (Tilman and Pacala 1993). Heterogeneity in soil resource factors generally fails to promote plant species diversity (e.g. Baer et al. 2005; Eilts et al. 2011; Gazol et al. 2013; Price et al. 2017; Reynolds et al. 2007; but see Baer et al. 2016) while soil heterogeneity in non-resource factors often has a positive influence on plant species diversity (e.g. Fitter 1982; Reynolds et al. 1997; Vivian-Smith 1997; Williams and Houseman 2013). The contrasting effects observed in studies where soil resource factors and non-resource factors have been manipulated could be due to the type of factors used, but these experiments also differ greatly in how long they were manipulated. Short-term diversity responses to heterogeneity are not comparable to long-term ones since community structures may change over time due to depletion of resources and expansion of plant species (Baer et al. 2005, 2016). So far, very few experimental studies have manipulated both soil resource and non-resource factors to test soil heterogeneity effects on plant species diversity (but see Baer et al. 2004, 2016).

Here, we report a three-year field experiment to test the effects of heterogeneity in different soil factors on plant species diversity at different spatial scales (grain size and focal scale). We manipulated two soil factors, i.e. soil nutrients and soil pH that are both considered to be important factors affecting the species composition of plant communities (Gough et al. 2012; Gough et al. 2000; Isermann 2005; Laliberté et al. 2014; Schaffers 2002; Tilman 1984, 1987). Soil nutrient availability and pH were manipulated separately. We sowed a seed mixture of 16 common grassland plant species in (i) heterogeneous soils consisting of low and high soil nutrients or pH patches that differed in patch size

(10 cm × 10 cm and 30 cm × 30 cm at which different levels of soil nutrients and pH were detected in the field), and in (ii) homogenous soils with low and high nutrient or pH soils evenly mixed. The experiment was carried out in poor sandy soils with naturally low nutrient availability and each plot was divided into 6 × 6 patches of 10 cm × 10 cm each, irrespective of the heterogeneity treatments. We only focused on plants in the central 40 × 40 cm of each plot to reduce edge effects, and performed analysis at two focal scales: 0.16 m^2 (40 cm × 40 cm plot-scale) and 0.01 m^2 (10 cm × 10 cm patch-scale).

We made the following predictions: (1) Plant species richness and diversity determined at both focal scales will be higher in plots where high and low nutrient or pH soils are patchily distributed (heterogeneous soil) than in plots where the two soils are homogeneously mixed (homogeneous soil), as soil heterogeneity is generally thought to promote plant species diversity (Tilman and Pacala 1993), independent of the focal scale (Lundholm 2009). (2) Species richness and diversity determined at both focal scales will be higher in plots with heterogeneous soil of large grain size (large patches) than in plots with heterogeneous soil of small grain size (small patches; Hutchings et al. 2003), because plants with large rooting systems can grow across patches when patch sizes are small, thus outcompeting inferior species. (3) Variation in species composition among patches (10 cm × 10 cm) will be greater in heterogeneous than in homogeneous plots because within the heterogeneous plots different soil patches will support distinct sub-communities.

Materials and methods

The experiment

In early spring 2015, original topsoil of an experimental field in the central part of the Netherlands (51°59′N 5°39′E) was removed to a depth of 90 cm and refilled with a 1:4 (v:v) mixture of black soil (collected from a former arable field) and yellow sand. We then pushed 30 wooden frames (60 cm wide × 60 cm long × 40 cm deep) into the soil to a depth of 35 cm. Each frame thereafter was referred to as a plot. The soil within each plot was removed and replaced by the experimental soils described below. The 30 plots were arranged in five blocks with each block containing 6 plots. The distance between adjacent plots was 0.9 m. The footpath between the plots was sown with a mixture seeds of the grasses *Poa pratensis* and *Lolium perenne*.

We manipulated two soil factors, i.e. soil nutrients and soil pH, separately in this experiment. For each soil factor, there were three treatments i.e., one homogeneous soil treatment and two heterogeneous soil treatments with different grain sizes (Fig. 1). The size of the patch was chosen because at this patch size different levels of soil nutrients and pH were detected in the field (Březina et al. 2019; Kreuzeder et al. 2018; Reynolds et al. 2007). In the homogeneous soil nutrient treatment, each plot was filled with a 2:2 (v:v) mixture of black soil and yellow sand. In the two heterogeneous soil nutrient treatments, each plot was equally divided into either 36 "small patches" (10 cm × 10 cm) or 4 "large patches" (30 cm × 30 cm). Each patch was filled with either low [1:3 (v:v) mixture of black soil and yellow sand] or high nutrient soil [3:1 (v:v) mixture of black soil and yellow sand] in a checkerboard manner (Fig. 1).

In the homogeneous soil pH treatment, each plot was filled with a 2:1:1 (v:v:v) mixture of black soil, yellow sand and cyclone sand with 72 g $CaCO_3$ (200 g/m^2). The amount of $CaCO_3$ supplied to the pH treatments was based on Elberse et al. (1983). The two heterogeneous soil pH treatments were created using low [1:1 (v:v) mixture of black soil and yellow sand] and high pH soils [1:1 (v:v) mixture of black soil and cyclone with 144 g $CaCO_3$ (400 g/m^2)] (Fig. 1). The total amount of nutrients in the homogeneous nutrient treatment and the two heterogeneous nutrient treatments, as well as the total amount of $CaCO_3$ in the homogeneous pH treatment and the two heterogeneous pH treatments were equal.

For each soil used in the experiment, we randomly took five soil samples for soil chemical analysis. Initial soil chemical characteristics are presented in Table 1. To ensure there was a distinct difference between the low and high pH soil, we added 2 g lime in each of the 18 high-pH patches within the two heterogeneous pH treatments. To make the homogeneous and heterogeneous soil pH treatments comparable, we also added 36 g lime to the plots with homogeneous soil pH treatment. In this way, the total amount of lime added to the homogeneous and heterogeneous pH soil was equal. This was done twice a year, i.e. early during the growing season and after the harvest at the end of the growing season.

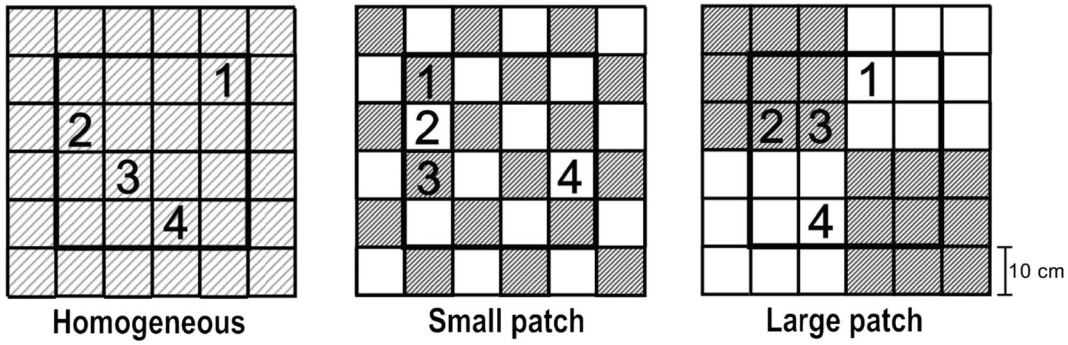

Fig. 1 Schematic representation of the experimental design. The experiment consisted of one homogeneous soil treatment with medium level of nutrient availability/pH and two heterogeneous soil treatments with low and high nutrient/pH soil patches and differing in patch size (small patches and large patches). Soil nutrients and soil pH were manipulated separately. See the main text for the soils used in each treatment. Aboveground biomass were harvested separately for all the central 16 patches (within the thick black square). Numbers mark the patches in which aboveground biomass were sorted to species (presented are the four randomly selected patches in homogeneous nutrient soil, heterogeneous nutrient soils with small and large patches in the first block)

Plant community

We used 16 common grassland species (graminoid, i.e., *Anthoxanthum odoratum*, *Briza media*, *Festuca rubra* and *Luzula campestris*, and forbs, i.e., *Achillea millefolium*, *Campanula rotundifolia*, *Centaurea jacea*, *Hypochaeris radicata*, *Knautia arvensis*, *Leontodon hispidus*, *Leucanthemum vulgare*, *Plantago media*, *Prunella vulgaris*, *Rumex acetosa*, *Veronica chamaedrys* and *Sanguisorba minor*) in this experiment. These species were selected because they vary in their optimal soil nitrogen and pH conditions in terms of Ellenberg values (Table S1; Ellenberg et al. 1992). To create the plant community, a mixture of 96 seeds (with six seeds of each species) was evenly sown in each patch (10 cm × 10 cm) within each plot. Seeds were purchased from Cruydthoeck, Nijeberkoop, The Netherlands. In total, we planted 3456 seeds in each plot (9600 seeds/m^2; a similar sowing rate applied as Wijesinghe et al. 2005). All species used in the experiment are native to the Netherlands and perennials with different growth forms and germination rates (Table S1). We did not include legume species because they can fix atmospheric N_2 (Trannin et al. 2000), which may potentially alter the nutrient availability and hence influence soil heterogeneity within plots. To introduce microbial communities, after sowing, the plots were evenly covered by

Table 1 Soil chemical analysis of different soils

Soil	N-NH_4 (mg/kg) ($n = 5$)	P-PO_4 (mg/kg) (n = 5)	N-NO_3 (mg/kg) (n = 5)	K (mg/kg) (n = 5)	pH (H_2O) ($n = 3$)	Organic matter (%) ($n = 2$)
Low nutrient soil	2.95 ± 0.29^{a}	0.35 ± 0.09	1.80 ± 0.16^{c}	22.64 ± 1.59^{b}	6.97 ± 0.01^{b}	0.98 ± 0.03^{c}
Homogeneous nutrient/Low pH soil	2.09 ± 0.62^{ab}	0.11 ± 0.04	5.55 ± 0.79^{b}	30.70 ± 2.73^{ab}	6.86 ± 0.02^{c}	2.09 ± 0.06^{b}
High nutrient soil	2.22 ± 0.41^{ab}	0.29 ± 0.13	9.25 ± 1.52^{a}	35.98 ± 1.70^{a}	6.52 ± 0.01^{d}	3.46 ± 0.05^{a}
Homogeneous pH soil	3.15 ± 0.44^{a}	0.16 ± 0.10	6.47 ± 0.68^{ab}	29.34 ± 3.57^{ab}	7.01 ± 0.04^{b}	2.19 ± 0.07^{b}
High pH soil	0.84 ± 0.07^{b}	0.19 ± 0.10	6.60 ± 0.52^{ab}	28.92 ± 2.62^{ab}	7.15 ± 0.00^{a}	2.37 ± 0.09^{b}
One-way ANOVA	4.95**	0.98	9.82***	3.50*	147.61***	190.86***

Means (±SE), sample size (n) and *F*-values of one-way ANOVA are given. Tukey post-hoc tests were made among the five soils, mean values sharing the same superscript (a-d) are not significantly different. Symbols give: *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$. The amount of N-NH_4, N-NO_3 and P-PO_4 (mg/kg dry soil sample) were determined by adding 30.0 ml of 0.01 mol/L $CaCl_2$ solution to soil samples (3.0 g), shaking mechanically for at least 2 h at room temperature (20 °C), filtering the solution and analyzing the nutrients in the soil extracts in a flow analyzer (SKALAR SAN plus system). Soil pH-H_2O was determined by adding 25.0 ml demi-water to soil samples (volume 5.0 ml), shaking for 5 min and measuring 2 h later. Soil organic matter was determined by measuring the difference between weights of the oven-dried (105 °C) soil samples (5.0–10.0 g) before and after being heated in a furnace at 550 °C

0.8 L of a 1:3 (v:v) mixture (sieved through 0.2 mm mesh) of live natural grassland soil (collected in a grassland two kilometres away from the experiment garden) and low nutrient soil.

To ensure the subsequent treatment effects on plant species diversity were not caused by initially unequal germination, we performed an additional experiment in an unheated greenhouse. In this experiment, seeds were sown in pots filled with the five soils used in the field experiment. After one month, we counted the species richness and calculated the species diversity. There were no significant differences among the soils (richness: $F_{4, 24} = 0.96$, $P = 0.449$; diversity: $F_{4, 24} = 0.76$, $P = 0.563$).

All weeds that emerged from the seed bank were removed by hand before sowing. After sowing, we weeded all plots at the beginning of each growing season. During the first three months of the experiment the plots were watered twice a day to promote the germination and establishment of the sown plant species. The experiment was maintained for three growing seasons (2015–2017). During the experiment the daily mean temperature and precipitation were 15.4 °C (range 7.5 to 26.2 °C) and 3.3 mm (range 0 to 26.0 mm) in 2015, 13.9 °C (range 0 to 25.2 °C) and 2.7 mm (range 0 to 66.6 mm) in 2016, and 13.6 °C (range 3.2 to 24.6 °C) and 2.1 mm (range 0 to 24.6 mm) in 2017, respectively (http://www.knmi.nl).

Harvest measurements

All aboveground biomass in the central sixteen 10 cm × 10 cm patches were harvested separately at the end of each growing season (on 18th September 2015, 12th September 2016 and 10th August 2017, respectively) by cutting the vegetation at 1 cm above soil level. We considered a plant to live in a given patch if it rooted within the patch, regardless of whether the leaves were inside or outside this patch. We randomly selected four patches (or two patches for each soil type in the heterogeneous plots) from the inner 16 patches of each plot (see an example in Fig. 1), all plant material from each of the selected patches at each harvest was sorted to species. To determine belowground biomass, at the final harvest, soil cores (4.5 cm in diameter, 40 cm deep) were taken from the same four randomly selected patches in each plot. The belowground parts were carefully washed over a sieve (0.5 mm mesh). Separation of roots by plant species was not possible. Aboveground biomass of each plant species in each patch and belowground community biomass in each patch was determined after oven-drying at 70 °C for at least 48 h.

Data analysis

Using the data from the four randomly selected patches within a plot, we determined plant species richness and diversity at two different focal scales: 0.16 m^2 plot-scale (40 cm × 40 cm; $n = 4$) and 0.01 m^2 patch-scale (10 cm × 10 cm; n = 4). Plot-scale species richness was determined by counting the total number of the species over the four sampled patches per plot. Plot-scale diversity (H') was calculated as: $H' = -\sum_{i=1}^{S} P_i \ln P_i$, where S is the plot-scale species richness and P_i is aboveground biomass of species i divided by total aboveground biomass of all plant species in the four sampled patches in a plot. We used biomass rather than the number of individuals to calculate H' to account for differences in the size of species in a community (Lyons 1981). Patch-scale species richness was determined by averaging the species number over the four sampled patches per plot (or in the case of heterogeneous soil treatments, averaged over each of the two patch types). Patch-scale diversity was determined by first calculating the diversity of each sampled patch using aboveground biomass of each species in each patch, then this value was averaged over the four sampled patches in a plot (or in the case of heterogeneous soil treatments, averaged over each of the two patch types).

We also determined plant species composition at the 0.16 m^2 plot-scale (40 cm × 40 cm) and at the 0.01 m^2 patch-scale (10 cm × 10 cm). For the plot-scale species composition, total aboveground biomass of each plant species in the four sampled patches per plot was used while for the patch-scale species composition, mean aboveground biomass of each plant species over the four sampled patches per plot was used. Plant species that occurred in less than 5% of the samples were excluded in the community composition analysis (McCune et al. 2002).

We determined the variation in species composition (beta diversity) within each plot (or in the case of heterogeneous soil treatments, within each type of soil patch). We first calculated a Bray-Curtis dissimilarity matrix based on square-root transformed aboveground biomass data. Then, we computed the mean pairwise Bray-Curtis dissimilarity between each pair of patches

within each plot (or in the case of heterogeneous soil treatments, within each type of soil patch) for each sampling year. These mean pairwise dissimilarities were used as the variation in species composition (beta diversity) within each plot (or in the case of heterogeneous soil treatments, within each type of soil patch).

We first tested the soil heterogeneity effects on plant community responses. Effects of soil heterogeneity in nutrients and pH were tested separately because their soil composition was initially different. We used a linear mixed-effects model to test the effects of soil heterogeneity treatment (homogeneous vs. small patch vs. large patch), time (2015 vs. 2016 vs. 2017), and their interaction on both plot-scale and patch-scale species richness and diversity, as well as mean Bray-Curtis dissimilarity among patches. As we sampled the same experimental plot during three consecutive years, plot was included as a random factor to account for the repeated measurements. Post-hoc comparisons among levels of the soil heterogeneity treatment were tested using planned contrasts across all three years, as well as for each year separately (Wubs and Bezemer 2016).

We used unconstrained, principal component analysis (PCA) to explore plot-scale and patch-scale plant community composition under different soil heterogeneity treatments. We further performed constrained redundancy analysis (RDA) to determine whether variation in patterns of the plot-scale and patch-scale plant composition could be explained by time and differences among the three soil heterogeneity treatments. In the RDA, year and soil heterogeneity treatment were used as explanatory variables. Significance was based on a permutation test (499 permutations) using a split-plot design. We took the three recordings of each treatment as "Whole plots" and the recordings as "Split plots" according to Canoco terminology. Split-plots were permutated within the whole plots, while the whole plots were not permuted (Lepš and Šmilauer 2003).

We performed linear mixed-effects models and computed Bray-Curtis dissimilarity metrics in R (version 3.3.2; http://www.r-project.org) and RStudio (version 1.0.44; http://rstudio.org). Linear mixed-effects models were fitted with the *nlme* package (version 3.1–128; Pinheiro et al. 2016) and all data were checked graphically for normality and homogeneity of variance. Model variance components were estimated using restricted maximum likelihood (REML) and the denominator degrees of freedom of *F*-tests were calculated following the inner-outer approach (Pinheiro and Bates 2006). Post-hoc comparisons were made as planned contrasts using the *multcomp* (version 1.4–8) package (Hothorn et al. 2008), and univariate uncorrected *P*-values were reported. Bray-Curtis dissimilarity metrics were calculated using the *vegdist* function in the *vegan* package (version 2.4–4) and all abundance data were root square transformed prior to analysis. All multivariate analyses were conducted in Canoco 5.03 (Microcomputer Power, Ithaca NY, USA).

Results

Species richness and diversity

Plot-scale (40 cm × 40 cm) species richness, diversity or evenness was not significantly different among the three soil nutrient heterogeneity treatments (Fig. 2a, b, S1A; Table 2A). The heterogeneous pH treatment also did not influence plot-scale species richness (Fig. 2c; Table 2B). However, the plot-scale diversity and plot-scale evenness was significantly greater in heterogeneous pH plots with large grain size than in heterogeneous pH plots with small grain size and in homogeneous pH plots, but this was only significant at the last harvest (Fig. 2d, S1C; Table 2B).

Soil nutrient heterogeneity significantly influenced the patch-scale (10 cm × 10 cm) species richness, as indicated by the lower patch-scale richness in heterogeneous nutrient soils, both with small and large grain sizes, than in homogeneous nutrient soil (Fig. 3a; Table 3A). However, the grain size of soil nutrient heterogeneity did not have a significant effect (Fig. 3a; Table 3A). Patch-scale diversity or patch-scale evenness did not show any significant responses to soil nutrient heterogeneity or its grain size (Fig. 3b, S1B; Table 3A). Soil pH heterogeneity did not influence patch-scale species richness (Fig. 3d; Table 3B). However, patch-scale diversity, consistent with patch-scale evenness was significantly greater in heterogeneous pH plots with large grain size than in heterogeneous pH plots with small grain size and in homogeneous pH plots at the final harvest (Fig. 3e, S1D; Table 3B).

Patch-scale richness and diversity were initially low and generally smaller than plot-scale richness and diversity (Figs. 2 vs. 3), indicating different alpha and gamma

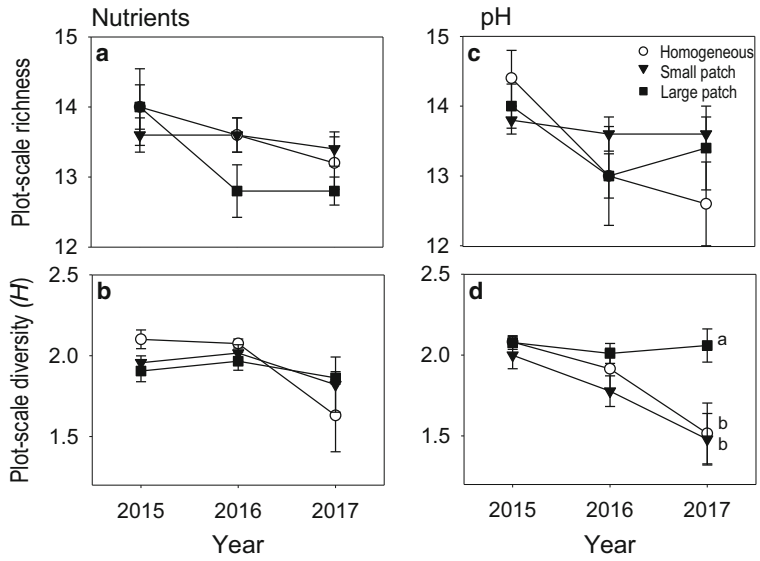

Fig. 2 Plot-scale (40 × 40 cm) species richness (**a** and **c**) and diversity (**b** and **d**) in the soil nutrient (left panel) and pH (right panel) heterogeneity treatments (i.e. the homogeneous soil, small patch and large patch heterogeneous soils) from 2015 to 2017. Mean values (±SE) are given. "Homogeneous", "Small patch" and "Large patch" represent homogeneous soil and heterogeneous soil with small and large patch sizes, respectively. See Table 2 for statistical results and post-hoc comparisons among levels of soil heterogeneity treatment overall across all three years. Post-hoc comparisons among levels of the soil heterogeneity treatments were made as planned contrasts for each year separately in each panel: means that share the same letter (a–b) within a year are not significantly different at $P < 0.05$

diversity. In general, species richness and diversity quantified both at plot-scale and patch-scale decreased in all treatments from 2015 to 2017 (Figs. 2 and 3; Tables 2 and3).

Table 2 Results of a mixed-effect ANOVA testing effects of time (2015 vs. 2016 vs. 2017), soil heterogeneity (homogeneous vs. small patch vs. large patch) and their interaction on plot-scale (40 cm × 40 cm) species richness and diversity

			Plot-scale richness			Plot-scale diversity (H')		
	DF	denDF	F	Z	P	F	Z	P
(A) *Soil heterogeneity in nutrients*								
Time (T)	2	24	4.04	–	**0.031**	5.67	–	**0.010**
Heterogeneity (H)	2	12	1.29	–	0.310	0.04	–	0.962
Homogeneous vs. small patch	–	–	–	−0.25	0.802	–	−0.05	0.964
Homogeneous vs. large patch	–	–	–	−1.50	0.134	–	−0.26	0.794
Large patch vs. small patch	–	–	–	1.25	0.211	–	0.22	0.829
T × H	4	24	1.04	–	0.406	1.46	–	0.245
(B) *Soil heterogeneity in pH*								
Time (T)	2	24	4.90	–	**0.016**	11.50	–	**<0.001**
Heterogeneity (H)	2	12	0.31	–	0.740	3.71	–	0.056
Homogeneous vs. small patch	–	–	–	0.78	0.435	–	−0.76	0.450
Homogeneous vs. large patch	–	–	–	0.31	0.755	–	1.89	0.059
Large patch vs. small patch	–	–	–	0.47	0.639	–	−2.65	**0.008**
T × H	4	24	1.33	–	0.286	2.87	–	**0.045**

F-values, *P*-values and degrees of freedom of a linear mixed-effects model, and *Z*-values and *P*-values of overall planned contrasts among soil heterogeneity treatments are presented. Values are in bold when $P < 0.05$

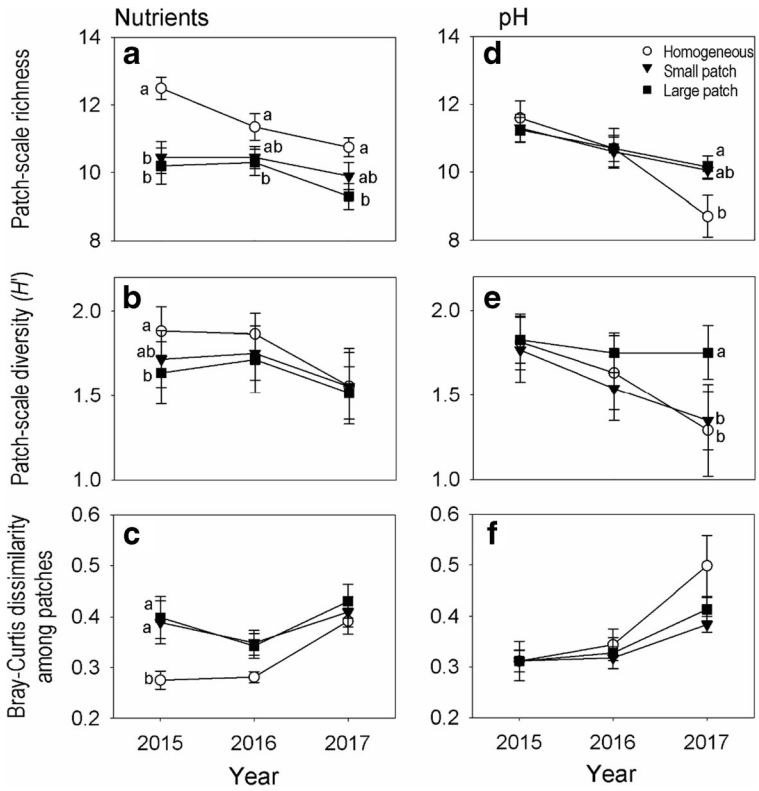

Fig. 3 Patch-scale (10 cm × 10 cm) species richness (**a** and **d**) and diversity (**b** and **e**), and mean Bray-Curtis dissimilarity among patches (**c** and **f**) in the soil nutrient (left panel) or pH (right panel) heterogeneity treatments (i.e. the homogeneous soil, small patch and large patch heterogeneous soils) from 2015 to 2017. Mean values (±SE) are given. "Homogeneous", "Small patch" and "Large patch" represent homogeneous soil and heterogeneous soil with small and large patch sizes, respectively. See Table 3 for statistical results and post-hoc comparisons among levels of soil heterogeneity treatment overall across all three years. Post-hoc comparisons among levels of soil heterogeneity treatment were made as planned contrasts for each year separately in each panel: means that share the same letter (a–b) within a year are not significantly different at $P < 0.05$

Species composition and its variation

There was a significant heterogeneity effect on plant species composition at both plot-scale and patch-scale (Fig. 4). Species composition measured both at plot- and patch-scale changed significantly during the experiment period (Fig. 4). *Hypochaeris radicata* was the dominant plant species (relative abundance: ~30%) in the first two years. In 2017, however, its relative abundance reduced to only ~10% while the abundance of *Centaurea jacea* (~30%) and *Leontodon hispidus* (~20%) increased.

Moreover, soil nutrient heterogeneity significantly influenced the variation in plant species composition (mean Bray-Curtis dissimilarity among patches). The mean Bray-Curtis dissimilarity was greater in heterogeneous nutrient soils than in homogeneous nutrient soil (Fig. 3c; Table 3A). This difference disappeared at the end of the experiment, indicating that the communities at different soils moved towards a similar state over time. However, there was no difference between the heterogeneous nutrient soils with small and with large patches (Fig. 3c; Table 3A), suggesting that the grain size of soil nutrient heterogeneity did not influence variation in species composition. The pH heterogeneity or its grain size did not influence the mean Bray-Curtis dissimilarity (Fig. 3f; Table 3B). The mean Bray-Curtis dissimilarity increased in all treatments from 2015 to 2017 (Fig. 3c, f), indicating that the composition of plant aboveground biomass significantly diverged from its initial composition in all soils.

Discussion

Our results show that a heterogeneous distribution of soil nutrients initially reduced plant species

Table 3 Results of a mixed-effect ANOVA testing effects of time (2015 vs. 2016 vs. 2017), soil heterogeneity (homogeneous vs. small patch vs. large patch) and their interaction on patch-scale (10 cm × 10 cm) species richness and diversity, and mean Bray-Curtis dissimilarity among patches

			Patch-scale richness			Patch-scale diversity (*H'*)			Mean dissimilarity [1]		
	DF	denDF	*F*	*Z*	*P*	*F*	*Z*	*P*	*F*	*Z*	*P*
(A) *Soil heterogeneity in nutrients*											
Time (T)	2	24	7.25	–	**0.003**	12.88	–	**<0.001**	10.30	–	**0.001**
Heterogeneity (H)	2	12	12.25	–	**0.001**	1.99	–	0.179	4.60	–	**0.033**
Homogeneous vs. small patch	–	–	–	−3.71	**<0.001**	–	−1.26	0.206	–	2.51	**0.012**
Homogeneous vs. large patch	–	–	–	−4.69	**<0.001**	–	−1.97	**0.049**	–	2.74	**0.006**
Large patch vs. small patch	–	–	–	0.98	0.329	–	0.71	0.480	–	−0.23	0.818
T × H	4	24	1.26	–	0.313	0.80	–	0.535	1.54	–	0.221
(B) *Soil heterogeneity in pH*											
Time (T)	2	24	16.66	–	**<0.001**	24.07	–	**<0.001**	28.35	–	**<0.001**
Heterogeneity (H)	2	12	0.25	–	0.781	3.06	–	0.084	0.43	–	0.658
Homogeneous vs. small patch	–	–	–	0.57	0.571	–	−0.28	0.778	–	−0.91	0.361
Homogeneous vs. large patch	–	–	–	0.66	0.512	–	1.99	**0.049**	–	−0.62	0.538
Large patch vs. small patch	–	–	–	−0.09	0.929	–	−2.27	**0.023**	–	−0.30	0.765
T × H	4	24	2.07	–	0.117	4.17	–	**0.011**	1.44	–	0.252

F-values, *P*-values and degrees of freedom of a linear mixed-effects model, and *Z*-values and *P*-values of overall planned contrasts among soil heterogeneity treatments are presented. Values are in bold when $P < 0.05$

[1] Data were ln-transformed

richness when compared to a homogeneous soil that has the same amount of total nutrients. However, a spatially patchy arrangement of soil pH increased plant species diversity compared to the corresponding homogeneous pH soil when the grain size of the soil pH heterogeneity was large, even though this was only true at the final harvest. These effects prevailed when species richness and diversity were determined at the patch scale but were rather weak when measured at the plot scale. Therefore, our study implies that spatial soil heterogeneity can influence plant species diversity (Bakker et al. 2003; Reynolds and Haubensak 2009), but depends on the type of soil factors that are manipulated (resources vs. non-resources), and also on the focal spatial scale and grain size of the soil heterogeneity.

In agreement with other experimental studies, heterogeneity in soil nutrient supply reduced plant species diversity (Eilts et al. 2011; Gazol et al. 2013). Previous studies suggested that plants may become dominant when their rooting systems exceed the grain size of the soil heterogeneity, as they may integrate resources across patches (Eilts et al. 2011; Fransen et al. 2001; Hutchings et al. 2003). We do not know how far the roots of the species extended horizontally in our study. However, half of the species in our experimental communities are clonal plants and they can exploit high nutrient soils through connecting rhizomes or stolons. As a result, high nutrient soil patches supported higher species richness (Fig. S2A) and community biomass (Fig. S3A, B) than low nutrient soil patches within the heterogeneous nutrient soils. This may have increased interspecific competition thus lower plant species diversity in the heterogeneous soils (Goldberg 1987). In contrast to what we hypothesized, the grain size of soil nutrient heterogeneity did not influence plant species richness or diversity. We suggest that this resulted from the small difference in grain sizes used in our study. It is likely that the plant species in our study can relatively outgrow both patch sizes.

Soil pH heterogeneity did not influence plant species richness, however, at final harvest, heterogeneous pH plots with large grain size had higher plant species diversity than heterogeneous pH plots with

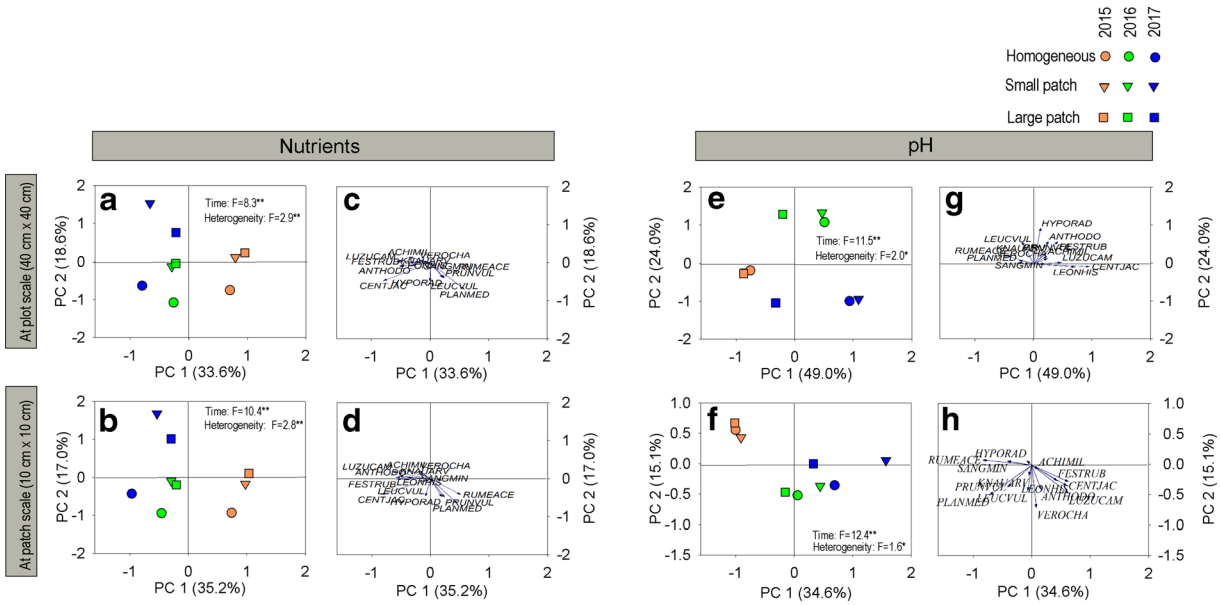

Fig. 4 Principal component analysis (unconstrained PCA) plots showing effects of soil heterogeneity on the distribution of aboveground biomass over the component plant species in different plant communities at both plot-scale (upper panel) and patch-scale (lower panel). Circles, triangles and squares represent mean sample scores for the homogeneous soil and heterogeneous soil with small and large patch sizes, respectively. Different colours separate mean sample scores for year 2015, 2016 and 2017, respectively. Statistics shown in each panel are *F*-statistic of constrained redundancy analysis (RDA) on time and soil heterogeneity treatment (Heterogeneity). Asterisks indicate significance: * $P < 0.05$ and ** $P < 0.01$. Abbreviations: ANTHODO-*Anthoxanthum odoratum*, FESTRUB-*Festuca rubra*, LUZUCAM-*Luzula campestris*, ACHIMIL-*Achillea millefolium*, CENTJAC-*Centaurea jacea*, HYPORAD-*Hypochaeris radicata*, KNAUARV-*Knautia arvensis*, LEONHIS-*Leontodon hispidus*, LEUCVUL-*Leucanthemum vulgare*, PLANMED-*Plantago media*, PRUNVUL-*Prunella vulgaris*, RUMEACE-*Rumex acetosa*, VEROCHA-*Veronica chamaedrys* and SANGMIN-*Sanguisorba minor*

small grain size and homogeneous pH plots. This supports the heterogeneity-diversity hypothesis (Ricklefs 1977; Tilman and Pacala 1993). One possible explanation for the distinct effects of soil pH heterogeneity on species richness and species diversity is that at the final harvest, plant community in heterogeneous pH soils of large grain size had higher evenness than that in heterogeneous pH soils of small grain size and homogeneous soils.

We expected a greater variation in species composition in heterogeneous soils than in homogeneous soil due to different sub-communities in different soil treatments. This was partly supported by the experimental results as we found that species composition varied more in the two heterogeneous nutrient soils than in the homogeneous nutrient soil. The higher variation in heterogeneous soils, however, is likely due to higher variation in the low nutrient soil patches, rather than distinct sub-communities in low and high nutrient soil patches (Fig. S4: no effect on species composition). In contrast, the three soil pH heterogeneity treatments had a similar pattern in species composition variation. This is likely because plant species composition and its variation were similar in low and high pH soil patches within the heterogeneous pH soils.

We expected no effect of focal scale (plot vs. patch) on richness and diversity response to soil heterogeneity (Lundholm 2009), but focal scale mattered for these responses to nutrient heterogeneity. In general, patch-scale plant species richness and diversity (averaged over patches) were initially low because different soil patches initially favoured different subsets of species, as indicated by the considerable variation in community composition. However, when species richness and diversity were quantified at the plot scale (summed across patches), all plant species recorded in different patches were pooled (Allouche et al. 2012; Rapson et al. 1997). This may have led to a higher number of species at the plot scale than at the patch scale, and a similar number of plant species quantified at plot scale in the three soil nutrient heterogeneity

treatments. This result indicates that soil nutrient heterogeneity effects on species richness may have been covered by the effects of focal scale.

The temporal increase in mean dissimilarity indicates that plant communities in all treatments significantly diverged from their initial composition. This may relate to stochastic events due to the small sample sizes, as the individual number of plants in a patch would decrease when the dominance of certain species increases over time (Kreyling et al. 2011; Li et al. 2016; Orrock and Watling 2010). Moreover, plant communities in all nutrient treatments tended to move towards a similar state as the influence of initial soil conditions declined with time (Li et al. 2016). In our study, plant communities converged to become increasingly dominated by *Centaurea jacea* and *Leontodon hispidus*.

We conclude that soil nutrient heterogeneity can reduce plant species diversity if certain species become dominant while soil pH heterogeneity can increase plant species diversity through equalizing the relative performance of the different plant species in the community. These two contrasting effects prevailed when species richness and diversity were quantified at a small focal scale, and these effects also changed over time. Our study highlights the importance of variation in soil characteristics and temporal-spatial scales in evaluating soil heterogeneity effects on plant species diversity. These results may have important implications for plant community restoration in the field. On the one hand, soil heterogeneity has the potential to promote plant species coexistence and support higher plant species diversity, hence it is possible to restore degraded vegetation by creating mosaic habitats. One the other hand, other practices such as mowing and grazing that increase plant similarity should also be applied to reduce the negative influences of soil heterogeneity in the restoration process (Collins et al. 1998; Baer et al. 2016). It should be noted that in the field, there is more variation in soil characteristics along a variety of temporal-spatial scales. The response of plant species diversity to soil heterogeneity may be distinct when two or more soil factors vary spatially (Farley and Fitter 1999; Baer et al. 2016). Moreover, soil heterogeneity for both levels in our experiment should be considered small-scale soil heterogeneity. We do not know how larger-scale soil heterogeneity will influence plant species diversity. Therefore, future studies testing soil heterogeneity-plant species diversity relationships should consider multiple soil characteristics across various temporal-spatial scales at which plant species diversity and soil heterogeneity are measured.

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