Seed sourcing for restoration measures is a currently highly debated issue in conservation ecology. Where to take the seeds from, if reintroduction of plant species is necessary? In many countries, seed transfer zones have been defined to preserve genetic variability and to provide locally adapted plants. However, delineation of seed transfer zones mostly relies on climatic gradients, ignoring other abiotic factors, such as bedrock and soil conditions. One of the main soil properties is soil reaction (pH), yet it is still unclear whether pH is an important driver for local adaptation in plants. In this study, we tested whether plants are adapted to soil reaction across five common grassland species. For each species, we sampled seeds from two provenances, and within each provenance from two populations—one coming from basic and one from acidic soil. We grew the plants in a common garden in an artificial substrate with manipulated pH, which allowed us to test for the effect of soil reaction, isolated from other soil properties. All species produced more biomass on acidic substrate, in most cases independently of their original soil and provenance. Only one out of five species showed weak local adaptation to soil reaction. The experimental substrate by far explained most of the biomass production in all species. This suggests that adaptation to soil reaction is rare and of low importance in common species of temperate grasslands.

**Key words:** ecological restoration, ecotype, local adaptation, seed transfer zones, soil pH

**Implications for Practice**
- Our results support the current practice of defining seed transfer zones based on geological and climatic factors, albeit more species need to be tested.
- Local adaptation to soil pH can largely be neglected because it does not seem to be of great importance, at least in temperate grasslands of central Europe.

**Introduction**
In intensively used cultural landscapes of Central Europe, ecosystem restoration often faces biotic constraints such as missing target species due to local extinction (Bakker & Berendse 1999; Hölzel et al. 2012). When the soil seed bank is depleted, the success of restoration measures depends on availability of populations of target species in close vicinity to the restoration site that could serve as diaspore source. Often, these source populations are missing or too far away, making it impossible for target species to recolonize the site. Restoration of species-rich grasslands is therefore often seed-limited (Kiehl et al. 2010), and (re)introduction of plant species therefore mandatory. Consequently, the demand for seeds has grown rapidly over the last few years (Mortlock 2000; Vander Mijnsbrugge et al. 2010; Broadhurst et al. 2015; Nevill et al. 2018).

However, populations of most species are often genetically differentiated, and this differentiation reflects adaptation to environment. Although seeds from non-local sources, for example commercial cultivars, are often much more affordable than local seeds, their introduction to natural ecosystems may cause problems because they are not well adapted to their environment. Plants from non-local sources may also negatively influence the gene pools of local populations because of introgression of non-local genes, which may lead to outbreeding depression (Keller et al. 2000; Edmands 2007; Byrne et al. 2011; Frankham et al. 2011; Hufford & Mazer 2012). Consequently, local plants often perform better than plants of other origins, a pattern called local adaptation (Leimu & Fischer 2008; Baughman et al. 2019). Local seeds are thus expected to ensure optimal restoration success and they are often the preferred source for restoring functional ecosystems (Kiehl et al. 2010). Especially in light of climate change, it will be crucial to preserve local genetic diversity in order to maximize evolutionary potential (Havens et al. 2015; Bucharova et al. 2017; Breed et al. 2018).

Author contributions: DR, NH conceived and designed the research; DR performed the experiments; DR, AB analyzed the data; DR, NH, AB wrote and edited the manuscript.

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To make local provenancing practicable, many countries have established a system of seed transfer zones, that is regions with rather homogeneous environment where it is assumed that transfer of plants within the region will not result in a significant reduction of fitness. These transfer zones, like now mandatory in Germany, are usually delineated by a combination of geographic distance, geomorphology, and climate, thereby often following mainly a climatic gradient. Many studies have shown that geographic distance is indeed a good predictor of genetic differentiation (Joshi et al. 2001; Krauss et al. 2013; Bower et al. 2014; Jørgensen et al. 2016; Bucharova et al. 2017; Durka et al. 2017; Bucharova et al. 2019). Yet, there might be genetic variation on a much smaller spatial scale if we assume that plants also adapt to other abiotic and biotic conditions, like microclimate, nutrient or water availability, soil type, microbial communities, mycorrhiza, and more (e.g. Göransson et al. 2009; Varga & Kytöviita 2010; Smith et al. 2012; Fazlioglu et al. 2017; Gibson et al. 2019). Yet these factors are hardly considered when sourcing seeds for restoration (Vander Mijnsbrugge et al. 2010; Breed et al. 2018; Gibson et al. 2019; Terés et al. 2019).

One of the important soil conditions that can vary on rather small spatial scale is the soil reaction, which is for many vegetation ecologists one of the most important factors forming natural habitats (Ellenberg & Leuschner 2010). Basic soils often host different sets of species than acidic soils (Lee 1999; Hawkesford et al. 2012), and populations of species that grow on both basic and acidic substrate may be adapted to the population-specific pH. So far, there is not much known about plant adaptation to soil pH and the results of the existing studies are not conclusive (Göransson et al. 2009; Göransson et al. 2011; Fazlioglu et al. 2017; Young et al. 2018). In this study, we focused on population adaptation to soil pH across five common species of temperate grasslands in a reciprocal transplant experiment in a common garden environment. For each species, we sampled seeds from two different provenances, and within each provenance from two populations, one with acidic soils and one with basic soils. We grew the plants in standardized substrate with manipulated low and high pH, which allowed us to test for the effect of pH independently of other soil properties. We hypothesize that plants grown in substrate with similar pH to their source soil perform better than plants grown in substrate with different soil reaction.

**Methods**

We collected seeds of *Achillea millefolium* L., *Campanula rotundifolia* L., *Cynosurus cristatus* L., *Knautia arvensis* (L.) Coult, and *Plantago lanceolata* L., abbreviated by genus name hereafter, from wild populations. *Achillea* and *Plantago* are widely distributed common grassland species occurring also in richer habitats, whereas the other three species are more strictly bound to poorer and drier soils, but not demanding toward the soil reaction.

The two sample sites, hereafter called provenances, Eifel and Diemeltal (Fig. 1), are about 250 km apart from each other, situated in the west of Germany (coordinates: 50.37393, 6.67602 and 51.44473, 8.87001). They are characterized by nutrient-
poor habitats comprising soils from both calcareous and acidic bedrock near to one another. From each population and species, we collected seeds by hand of about 50 individuals from the base-rich (soil pH = 6.8) and the acidic soils (soil pH = 4.5–5, Table S1, Supporting Information). Each habitat measured about 20–30 ha. The two populations were separated by about 5–15 km, respectively. They were situated in the same seed transfer zone and had similar exposition, altitude, and thus, similar climatic conditions. The seed collection was carried out in 2016 by one single person. A sample of all fruiting individuals of the searched species was collected without regard to phenotypic traits, such as plant height.

We took the seeds to Münster, which is also situated in the west of Germany, and stored them over winter in a cool and dry place. To break dormancy, we put the seeds into the fridge at 5°C for 2 weeks and subsequently planted them in a greenhouse using a standard germination substrate made from 70% fine-grained white peat and 30% clay (pH 5.8) in April 2017. The germination substrate was used to ensure germination of enough plant individuals, as germination is likely to be inhibited under extreme conditions. To test the adaptation to pH, we obtained a special potting substrate with manipulated low and high pH (pH 3.96 and 6.24, respectively, Table S1). Both substrates consisted of 50% white peat, 20% coconut fiber, 10% wood fiber, 5% clay, and 5% lava. The pH was manipulated by adding carbonate to the peat-based, acidic substrate for the basic version. Both substrates contained similar amounts of nutrients, yet the nutrient content was considerably higher than in the field (Table S1). Using standardized substrate allowed us to test the effect of pH independently of other soil properties.

After 4–8 weeks, when the emerging seedlings had developed their first true leaves, we transplanted them into individual pots. All individuals of one species were transplanted in a very short period of time of not more than 2 weeks. The pots were filled with the prepared experimental substrate and placed outdoors in a common garden in a fully randomized design. From each of the two source populations per provenance, eight individuals were potted into low pH substrate and eight into high pH substrate (Fig. 1). In summary, the experiment comprised of eight seedlings per planting substrate, two planting substrates per populations, four populations (two per provenance) of five species, in total 320 plants (Fig. 1).

The plants were watered regularly during the whole season, but otherwise not treated. Pots were placed in a randomized pattern and switched regularly to avoid effects of differing light conditions. In autumn 2017, when the majority of plants had completed their growth cycle and started to die back, plant material was harvested species-wise. For determining biomass, we cut all aboveground parts of each plant, dried it at 70°C for 12 hours and weighed it subsequently. We chose biomass as commonly used proxy of fitness, because not all plants reached flowering stage during the experiment, and it was the only fitness proxy that was possible to use for all plants.

\[ \text{Results} \]

In four out of five species, the models explained 48–60% of variability in biomass production. The only exception was *Cynosurus* with 14% of explained variability (Fig. 2). The strongest effect on plant growth had the experimental substrate that explained up to 45% variability in the data. Irrespective of provenance or source soil, most species grew better on acidic substrate (Fig. 3, 1st column; Fig. 2). The strongest effect of substrate was detected in *Plantago* and *Achillea*, where it explained more than 40% of the variation in the biomass production. The only exception was *Knautia*, where the slope of the reaction differed between the provenances (Figs 2 & 3). For *Knautia*, however, the interaction of substrate and provenance was highly significant instead, accounting for nearly 30% of the variance.

The pH of the original soil at the source populations affected plant growth in four of five species (Fig. 3, 2nd column). Yet, the direction was species-dependent and had much weaker explanatory value than the experimental substrate by explaining only 5–
10% of the variance (Fig. 2). Only for *Campanula* the source soil played a larger role in interaction with provenance by accounting for 33% of the variation in the data. Provenance was further an important predictor of growth of *Knautia*, especially in interaction with planting substrate (Fig. 2; Fig. S1).

Local adaptation to soil reaction is represented by the interaction of the reaction of source soil and planting substrate. After correcting the model for all other variables, we detected local adaptation only in one species, *Achillea*, and it explained only 3% of variability (Fig. 2; Table S2).

**Discussion**

**Effect of the Experimental Substrate’s pH**

We found that most plants produced more biomass on low pH substrate. This trend was mostly independent of the soil reaction at the source of the planting material.

We ascribe that to a changed nutrient availability in the experimental substrate.

A species’ preference for a certain soil reaction correlates with preference for certain nitrogen forms (Bartelheimer &...
more or less parapatric distribution in Central Europe (Ehrendorfer 1962; Kolář et al. 2009; Kolář et al. 2012; Durka et al. 2017). In Campanula, the effect of provenance was driven by poor performance of one of the provenances. A possible explanation is that the source population was rather small and the plants could have suffered from inbreeding and negative effects of genetic drift (Fischer & Matthes 1998).

Effect of Local Adaptation (Soil × Substrate)

As the soil pH is considered to be one of the most important environmental factors determining plant communities (Ellenberg & Leuschner 2010), we expected that plants would be adapted to it and perform better when planted into substrate with similar pH value to their original soil. However, this was mostly not the case. The employed experimental substrate explained much more of the plants’ performance than the substrate x source soil interaction. Only one out of five species, Achillea, showed a significant interaction, yet only with weak explanatory value in comparison to other factors. Albeit not significant, the other four species rather showed a maladaptation in several cases, contradicting our hypothesis that plant individuals are locally adapted to soil pH. These results are in line with the inconsistent results of the few available studies on plant local adaptation to soil pH. Weak local adaptation was found in experiments that focused on abiotic conditions connected with pH such as elevated aluminum or carbonate concentration, rather than on the effect of pH itself (Göransson et al. 2009; Teréš et al. 2019). On the other hand, studies that focused solely on pH did not find any genetically underpinned adaptation, which suggests that phenotypic plasticity may be the most important mechanism for how plants cope with pH differences (Göransson et al. 2011; Fazlioglu et al. 2017).

An important effect of soil pH is nutrient availability for plants, with nutrients being generally less available under high pH conditions (White & Broadley 2003). Our planting substrate contained more nutrients than natural soils, so it is possible that we could not detect local adaptation because the plants were not nutrient-limited. However, this was not the case. The amount of nutrients remaining in the substrate after 1 year of plant growth was much higher in the basic than in the acidic substrate. This indicates that the nutrients in basic substrate were less accessible for those plants than on the acidic substrate and the plants could not consume them. Thus, despite high total nutrient content, the plants have been nutrient-limited at the basic substrate.

Before broader generalizations can be drawn, we see a strong need for further research on local adaptation to soil conditions on the small scale, including in situ transplant experiments, as there is still little knowledge on the importance of various soil-related ecological interactions. Our results support the current delineation of seed transfer zones based on geological and climatic factors. As far as we can tell from these first investigated species, local adaptation to soil pH can largely be neglected because it does not seem to be of great importance, at least in temperate grasslands of central Europe. To justify this, more research on additional species is needed.
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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Boxplots showing biomass production per species, provenance and original soil in dependence of the experimental substrate.

Table S1. pH-values, P (phosphorus) and K (potassium) contents with respective standard errors of the original.

Table S2. ANOVA outputs for determinants of biomass production soil and the experimental substrate.

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