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Selection in response to community diversity alters plant performance in newly assembled test communities

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Abstract. In grassland biodiversity experiments the positive biodiversity–ecosystem functioning relationship generally increases over time. However, there is still a large gap in our understanding of the underlying short-term evolutionary processes. Research has shown that differential selection in monoculture vs. mixed-species communities can lead to rapid evolution. We assessed whether selection history altered productivity, biodiversity effects and species complementarity within newly assembled monocultures and 2-species mixtures using five plant species selected for twelve years in such a biodiversity experiment in mixture or monoculture and plants without such a selection history. Plants without past community selection history produced the lowest community biomass and showed the weakest biodiversity effects. Furthermore, we found that twelve years of selection history in monocultures or species mixtures differentiated plants into monoculture- and mixture-types within species. In newly assembled mixtures, plants with a selection history in mixtures performed better than plants with a monoculture selection history. Biodiversity effects were generally positive but, contrary to expectation, not stronger for mixture types. In addition, biodiversity effects were both influenced by trait differences among plants and community-weighted means, but these relationships were largely independent of selection history. Our findings indicate possible mechanisms underlying the rapid evolution of adapted subtypes within a species in grasslands. Uncovering these mechanisms contributes to our understanding of the biodiversity–ecosystem functioning relationship, which has the potential to influence species conservation practice.

Key words biodiversity effects, complementarity effect, ecosystem functioning, grasslands, plant productivity, sampling effect, species selection, trait variation

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INTRODUCTION

The loss of biodiversity due to species extinctions is a major threat to global ecosystems (Steffen et al. 2015) and has led to a large body of research investigating the importance of biodiversity to maintain ecosystem functions, such as productivity or nutrient cycling (Cardinale et al. 2012). In grasslands, many studies have found a positive biodiversity–productivity relationship (e.g. Tilman et al. 2001, Isbell et al. 2011), with biodiversity increasing multiple ecosystem functions (Soliveres et al. 2016) and ecosystem services (Balvanera et al. 2006). The positive effect of biodiversity has also been shown to increase with time (Cardinale et al. 2007, Reich et al. 2012), suggesting that complementarity between the co-occurring species can increase over time (Fargione et al. 2007).

Despite more than a decade of research on the biodiversity–productivity relationship (e.g. Reich et al. 2012), little is known about evolutionary mechanisms potentially affecting species interactions (Thorpe et al. 2011). It is conceivable that selection acting on traits could increase ecological combining ability (Harper 1977, Aarssen 1983) via niche differentiation in plant mixtures (Zuppinger-Dingley et al. 2014). Such adaptation may occur when there is either sufficient standing genetic variation in a population and the most suitable genotypes are sorted out (Fakheran et al. 2010) or by recombination and novel mutations (Anderson et al. 2011). Furthermore, plants may adapt to a novel environment by phenotypic plasticity (Price et al. 2003, Turcotte and Levine 2016), thus changing their morphology without genotypic changes. Epigenetic mechanisms have been suggested to enable adaptation (Bossdorf et al. 2008), especially in short-term evolutionary processes.

Whereas the influence of environmental factors on adaptive responses of plant populations is well studied (e.g. Schmid 1985, Joshi et al. 2001), much less effort has been devoted to studying the influence of community diversity on a species' performance (but see

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Lipowsky et al. 2011, Kleynhans et al. 2016). Based on previous observations in experimental ecosystems suggesting a "division of labor" among species in plant mixtures, it is likely that community diversity plays a role in the evolution of plant functional trait variation. For example, in forests more diverse tree communities have been shown to express greater crown complementarity (Niklaus et al. 2017, Williams et al. 2017). In diverse grassland communities, increased complementarity effects as estimated by the additive partitioning method of Loreau and Hector (2001) have been observed promoting community productivity via a range of mechanisms: diversification of the canopy structure and hence light and space use (Spehn et al. 2000, Allan et al. 2011), soil resource partitioning (Fornara and Tilman 2008, Roscher et al. 2008, von Felten et al. 2009), root depth distribution (Mueller et al. 2013) and distribution of leaf mass (Wacker et al. 2009). It is now timely to ask how and on what time scale selective forces may contribute to the evolution of the observed combining ability.

Using the additive partitioning method (Loreau and Hector 2001), net biodiversity effects (NEs) can be partitioned into complementarity (CEs) and sampling effects (SEs). When CEs drive over-yielding, most species are expected to contribute to greater biomass in more diverse communities. In contrast, when SEs are drive over-yielding, a few dominant species increase community productivity in species mixtures. The CE is therefore related to coexistence and trait variation between species, as it inherently suggests a differentiation in functional traits (Cadotte et al. 2009, Flynn et al. 2011). Conversely, the SE should rather be driven by traits of the dominant species and thus by community-weighted trait means (CWMs); an increase in CWMs (e.g. taller plants) should increase biodiversity effects (Roscher et al. 2012).

The use of functional traits to define species' niches has a long history in evolutionary ecology (van Valen 1965, Schoener and Gorman 1968, Roughgarden 1974) but

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only recently has become a popular approach in functional ecology (Violle et al. 2007) where it is being used to explain mechanisms of species coexistence and ecosystem functioning (Kraft et al. 2015, Hart et al. 2016). However, there is still a large gap in our understanding of how evolutionary mechanisms shape such trait-based niches (Roscher et al. 2015) and how they may drive corresponding niche differentiation according to functional traits (Sterck et al. 2011).

In particular, the selective power of community diversity on biodiversity effects as well as trait means and variation has received limited attention (but see Lipowsky et al. 2011, Zuppinger-Dingley et al. 2014, 2015, 2016, Kleynhans et al. 2016, Rottstock et al. 2017). Kleynhans et al. (2016) observed adaptation to new environmental conditions but only when the diversity level of the selection treatment and the assay treatment were the same. Although a selective past of growth in different diversity levels has been shown to have trans-generational influences on productivity for one species (Rottstock et al. 2017), it is unknown whether such an effect may be common to many species in plant communities.

In the present study, we tested whether community diversity as a selective environment can influence heritable variation in plant aboveground biomass and functional traits within and between species and how this may relate to biodiversity effects in two-species mixtures. We measured biomass production and traits of individual plants in monocultures and mixtures established with seedlings from either a selection history of experimental monoculture or mixture communities in a biodiversity field experiment (Jena Experiment, see (Roscher et al. (2004) for methods) or in monoculture fields from the commercial seed supplier which provided the original seeds for the biodiversity experiment in 2002. We refer to the plants growing in Jena since 2002 in mixture or monoculture experimental plots as mixture types and monoculture types, respectively. The plants derived from seeds obtained from the commercial supplier in 2014 are referred to as naïve plants.

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Whereas selection outcomes in an earlier study in the Jena Experiment were assessed after eight years and one controlled sexual reproduction cycle (Zuppinger-Dingley et al. 2014), in the present study we continued the selection treatment for another four years and added a second controlled sexual reproduction cycle. We included naïve plants as a control treatment without selection under continuous growth in monoculture or mixture communities. We hypothesized that during the twelve years of selection in the experimental field, mixture-type plants should have evolved high mixture performance (hypothesis 1, see Table 1). This should be related to large NEs, in particular CEs (hypothesis 2), and large between-species trait variation (hypothesis 3). Conversely, we hypothesized that monoculture-type plants should have evolved high monoculture performance (hypothesis 4), which should be related to large within-species trait variation (hypothesis 5). For control plants, we hypothesized intermediate results between monoculture- and mixture-type plants. We therefore aimed to expand on the relationship between biodiversity effects and between-species trait variation, hypothesizing that large CEs should be due to between-species trait variation (hypothesis 6). Finally, we hypothesized that large SEs should be due to large CWMs (hypothesis 7).

METHODS

Plant selection histories

To test whether plant types selected over twelve years in mixtures outperform those types selected in monocultures when assembled in mixture test communities, we chose five species grown in monoculture and mixture plots in the Jena Experiment (Jena, Thuringia, Germany, 51˚N, 11˚E, 135 m a.s.l., see Roscher et al. (2004) for experimental details): *Plantago lanceolata* L., *Prunella vulgaris* L., *Veronica chamaedrys* L., *Galium mollugo* L. and *Lathyrus pratensis* L. For brevity, we will use the genus names to refer to the species.

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The study species had previously been classified into the following functional groups (Roscher et al. 2004): *Veronica*, *Prunella* and *Plantago* as small herbs, *Galium* as a tall herb and *Lathyrus* as a legume.

Plant progeny from three different selection histories was used for the experiment. Plants without selection history in the Jena Experiment (selection history "naïve") were obtained from a commercial seed supplier (Rieger Hoffmann GmbH, Blaufelden-Raboldshausen, Germany). Plants with a selection history in the Jena Experiment were grown in either mixtures or monocultures from 2002 (selection history "mixture" and "monoculture", respectively). In 2010, cuttings of these plants were brought to Zurich and used for seed production for an earlier experiment. The plants were grown in their respective community in an experimental garden in Zurich and seeds were collected from these plants throughout the growing season of 2010. The propagation of seedlings from these seeds is described in Zuppinger-Dingley et al. (2014). These seedlings were then planted back into the experimental plots in Jena in 2011 using the identical parental species composition (for detailed procedure see van Moorsel et al. (2017)).

To ensure a second sexual reproductive event and to collect seed material for the present study, entire plant communities from some of the experimental plots replanted in Jena in 2011 were excavated again in March 2014. These plants were used to establish plots with an identical plant composition to the plots in Jena from which the plants were collected, in an experimental garden in slug-exclosure compartments at the University of Zurich, Switzerland (47°33′N, °37′E, 534 m a.s.l.). We added a layer of soil ("Gartenhumus" consisting of 50% agricultural soil and 50% garden compost, Ricoter, Aarberg, Switzerland) to each plot to ensure the plants established well. Mesh fabric netting around each plot minimized the possibility of cross-pollination between the same species from different experimental plots. Seeds were collected throughout the growing season of 2014 from

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monoculture plots and from 4- and 8-species mixtures. Seeds from different mother plants were pooled together. Seeds were cleaned manually for three species and mechanically for two species (*Plantago* and *Prunella*). The dry seeds were stored at 5° C for cold stratification until germination.

Experimental set up

Seeds were germinated in germination soil ("Anzuchterde", Ökohum, Herbertingen, Germany) under constant conditions in the glasshouse without additional light in December 2014, each species being sown on the same day. From 25 February to 13 March 2015, seedlings were planted in monocultures of four individuals and 2-species mixtures of four individuals into pots (two liters) filled with neutral agricultural soil (50% agricultural sugar beet soil, 25% perlite, 25% sand; Ricoter AG, Aarberg, Switzerland). Seedlings which died in the first two weeks were replaced with seedlings of the same species and age.

These seedlings were used to assemble test communities in six blocks (replicates) with each block consisting of the full experimental design as far as possible. Within each block, pots were placed on three different tables in the glasshouse in a randomized fashion without reference to selection history or assembly treatment. Throughout the experiment, we did not move pots but noted their position in the glasshouse. Single pots always contained four plants of the same selection history. Every selection history × species assembly combination was replicated five to six times depending on plant availability. We planted 30 monoculture and 42 mixture assemblies with mixture selection history, 30 monoculture and 60 mixture assemblies with monoculture selection history and 24 monoculture and 35 mixture assemblies with naïve selection history. There were thus 221 pots and 884 plants (Appendix S2 for monoculture identities and species combinations).

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During the experiment, we grew the plants initially at day temperatures of 17–20°C and night temperatures of 13–17°C without supplemental light. To compensate for overheating in summer, an adiabatic cooling system (Airwatech; Bern, Switzerland) was used to match inside with outside temperatures. The plants were not fertilized. Due to an infestation of white flies (*Trialeurodes vaporariorum*, Westwood 1856) and spider mites (*Tetranychidae* spp., Donnadieu 1875), we applied the insecticide SanoPlant Neem (1% Azadirachtin A (10 g/l); Maag AG) three times. The fungicide Fenicur (*Oleum foeniculi*, Andermatt Biocontrol) against powdery mildew (*Podosphaera* spp.) was applied twice. Plant height, leaf thickness, specific leaf area (SLA) and individual aboveground biomass were measured after twelve weeks of the experiment from 18 May to 4 June 2015. Leaf thickness was measured for three representative leaves using a thickness gauge. Specific leaf area (SLA) of up to 20 representative leaves (depending on the leaf size of the species) of each species in a pot was measured by scanning fresh leaves with a Li-3100 Area Meter (Li-cor Inc., Lincoln, Nebraska, USA) immediately after harvest and determining the mass of the same leaves after drying. Plant height and individual aboveground biomass were measured a second time after 24 weeks, the end of the experiment, from 18–25 August 2015. All four individuals in a pot were sampled. Research assistants, who were not informed of the specific experimental treatments, assisted in the regular measurements and harvesting of plants at the end of the experiment.

Data analyses

SLA outliers (> 99% percentile) were replaced with a maximum value (the 99% percentile, n = 6). We calculated pot-wise aboveground community biomass (plant community production) as the sum of the biomass of the four individual plants. Relative between-species differences (RDs, absolute difference between two species divided by the

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mean of the two) in plant height (first and second harvest), leaf thickness (first harvest) and SLA (first harvest) were calculated for mixture assemblies. Relative differences within species were calculated for both mixture and monoculture assemblies taking the relative difference between two individuals of the same species per pot. Furthermore, we calculated community-weighted means (CWMs) and pot standard deviation (SDs) for the same traits. Pots with dead plant individuals were excluded from the calculation of community-weighted means, but were included for the other data analyses. Net biodiversity effects (NEs) were calculated by comparing the 2-species mixtures with the average monoculture and partitioned according to Loreau and Hector (2001) into complementary (CEs) and sampling (selection) effects (SEs). This partitioning approach allows assessing how CEs and SEs contribute to the observed NEs (Loreau and Hector 2001). To avoid confusion with the term selection used for the selection-history treatment, we here use the term “sampling effect” for the SE (as in Zuppinger-Dingley et al. (2014)). Additive partitioning calculations were based on the difference between the observed yield of each species in the mixture and the monoculture yield for that species and selection history averaged across blocks. Absolute values of CE and SE were square root-transformed and the original signs put back on the transformed values for analysis (Loreau and Hector 2001). Differences in these measures between mixtures assembled from plants with monoculture selection history and mixtures assembled from plants with mixture selection history would suggest differential evolution of trait-based niches between species as a potential mechanism underlying biodiversity effects.

All statistical analyses were done in R (Version 3.2.3, R Core team 2016). Mixed-model analysis was done using the R-package asreml (VSNI international, 2016) and results assembled in ANOVA tables. Fixed-effects terms were selection-history treatment (naïve, monoculture, mixture), assembly treatment (monoculture vs. 2-species mixture assemblies), species identity of monoculture assemblies and of mixture assemblies (in short “species

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assembly") and interactions of these. Table (including blocks) was used as random-effects term. CWMs, RDs, within species differences and SDs of plant height, SLA and leaf thickness were added as covariates to models to investigate the influence of these covariates on community biomass and biodiversity effects.

RESULTS

Plant selection history and community productivity

We compared the community productivity of plants from different selection histories (naïve, monoculture, mixture) grown in newly assembled monocultures and 2-species mixtures by harvesting aboveground biomass twice, once after twelve weeks and a second time after 24 weeks. Because the first measure assessed growth and the second regrowth, the harvests were analyzed separately. Communities consisting of plants with naïve selection history produced the lowest community biomass at both the first and the second harvest (Fig. 1, Table 2). At the second harvest, this contrast between plants with and without selection history was stronger in mixture than in monoculture assemblies (Fig 1; interaction monoculture vs. mixture × naïve vs. monoculture or mixture in Table 2). Hence, both plants with monoculture- (unexpected) and with mixture- (expected) selection history in the Jena Experiment benefitted more from growing in mixtures (see also analysis of biodiversity effects in the next section).

At the second harvest the mixture-selection-history communities outperformed the monoculture-selection-history communities and this effect was marginally more pronounced in mixture assemblies (see Fig.1 and main effect mono types vs. mix types and the two-way interaction monoculture vs. mixture assembly × mono vs. mix types in Table 2). This partly confirms hypothesis 1 but not hypothesis 4 (see hypotheses listed in Table 1).

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Species identity in monoculture or mixture assemblies strongly influenced community productivity and, especially at the first harvest, the interaction terms with selection history were significant (main effect monoculture identity or species assembly of mixture and two-way interactions species assembly × naïve vs. mono or mix types and species assembly × mono types vs. mix types in Table 2). For example, at the first harvest, mixture-type plants performed better than monoculture-type plants in newly assembled monocultures of *Prunella* (rejecting hypothesis 4) and in mixtures of *Galium* and *Prunella* (confirming hypothesis 1) (Fig. 1a). However, in the two mixtures with the small herbs *Veronica* and *Prunella* and *Plantago* and *Prunella*, monoculture-type plants performed better than mixture-type plants (rejecting hypothesis 1; see Fig. 1a).

Plant selection history and biodiversity effects

Overall, biodiversity effects were positive at both harvests (First harvest: NE: $F_{1,15.9}$ = 26.67, $P < 0.001$, CE: $F_{1,15.8}$ = 8.214, $P = 0.011$, SE: $F_{1,14.2}$ = 97.07. $P < 0.001$, second harvest: NE: $F_{1,15.1}$ = 14.35, $P = 0.002$, CE: $F_{1,14.5}$ = 4.108, $P = 0.061$, SE: $F_{1,15.1}$ = 11.66, P = 0.004, Fig. 2, Appendix S3 and S4). At the first harvest, communities of naïve plants on average showed larger SEs than communities of selected plants ($F_{1,104.2}$ = 12.66, $P = 0.001$, Appendix S3). At the second harvest, however, NEs and CEs were significantly lower for naïve plant communities (NE: $F_{1,96.1}$ = 11.54, $P < 0.001$, CE: $F_{1,96.6}$ = 5.668, $P = 0.019$, Appendix S4). These results are in line with the results obtained for community productivity: plant communities consisting of plants without selection history had the lowest average productivity mainly because they could profit the least from growing in 2-species mixtures rather than in monocultures. We had expected naïve plants to have intermediate biodiversity effects between monoculture- and mixture-type plants.

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Contrary to our expectation (hypothesis 2), at the first harvest NEs, CEs and SEs were significantly larger for communities assembled from monoculture-type plants than for communities assembled from mixture-type plants (NE: $F_{1,93.9} = 21.01$, $P < 0.001$; CE: $F_{1,94.4} = 14.2$, $P < 0.001$; SE: $F_{1,101.2} = 10.28$, $P = 0.002$; Appendix S3; Fig. 2a–c, upper panels). This difference was reversed for most species assemblages at the second harvest (Fig. 2a–c, lower panels), when NE, CE and SE were non-significantly larger for communities assembled from mixture-type plants (Appendix S4). In line with the results obtained for community productivity, the influence of selection history on biodiversity effects also additionally depended on the specific species combination in mixture assemblies as follows (interactions species assembly × naïve vs. mono or mix types and species assembly × mono types vs. mix types in Appendix S3 and S4). At the first harvest, we found the expected result (hypothesis 2), i.e. a larger NE for mixtures types, for the combinations of *Galium* with either *Prunella* or *Plantago* (Fig. 2a, upper panel). At the second harvest, NEs and CEs were generally more similar between selection histories across different combinations and variation between the specific community compositions was mainly due to different SEs. An exception was the combination *Galium* + *Prunella*, which similarly to the first harvest showed a much larger NE for mixture-type plants, as expected under hypothesis 2. When both harvests were considered, communities including the legume *Lathyrus* or the small herb *Plantago* showed positive biodiversity effects (Fig. 2; effects of species assembly in Appendix S3 and S4). When comparing the CEs between the first and the second harvest, we found that four species combinations shifted from stronger biodiversity effects for monoculture types (rejecting hypothesis 2) to stronger biodiversity effects for mixture types (supporting hypothesis 2) (Fig. 2b). The *Galium* + *Prunella* species combination showed a consistently larger CE for mixture-type plants (supporting hypothesis 3). At the second harvest the different species combinations varied strongly in SEs, but not in CEs (CE: $F_{9,98.4}$

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= 1.121, $P = 0.356$, SE: $F_{9,100.8} = 11.53$, $P < 0.001$, Appendix S4). SEs were often larger for mixture than for monoculture types (Fig. 2c).

Plant selection history and within- and between-species trait variance

For SLA, plant height (at the first and at the second harvest) and leaf thickness we calculated relative differences within and between species as well as the total pot standard deviation (SD) and tested for differences between two of the three selection-history treatments (contrast between mixture- and monoculture-type plants, Fig. 3). The difference in plant height at the first harvest was marginally greater interspecifically for plants selected in mixtures, in accordance with hypothesis 3. In contrast to this hypothesis, the interspecific relative difference in leaf thickness was greater for plants selected in monocultures. Monoculture types showed greater intraspecific relative difference in SLA, in accordance with hypothesis 5. Furthermore, pot-level SDs in monocultures (were it was expected under hypothesis 5) or mixture assemblies were non-significantly larger for communities assembled with monoculture than with mixture types (see left two columns in Fig. 3).

Relationship between biodiversity effects and plant functional traits

We tested how the biodiversity effects were related to the measured functional trait variation (hypothesis 6; Fig. 4 and Fig. 5) and their means (hypothesis 7; Fig. 6 and Fig. 7). We calculated community-weighted means (CWMs) for plant height, SLA and leaf thickness at the first harvest and for plant height at the second harvest. We then related these CWMs to the partitioned biodiversity effects and analyzed if and how selection history could influence this relationship.

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First, we looked at the relationship of biodiversity effects with between-species differences (RDs) for SLA, plant height and leaf thickness in mixture pots (Fig. 4). The NE was negatively correlated with the RD of plant height and positively correlated with the RD of leaf thickness (see Fig. 4). Thus, while biodiversity effects decreased with increasing variation in plant height, they increased with increasing variation in leaf thickness. Contrary to our expectations under hypothesis 6, this was mainly driven by the SE, whereas the CE was less influenced by the RDs. Selection history had an effect on the relationship between biodiversity effects and RDs marginally or significantly. SEs were more negatively correlated with the RDs of plant height for mixture- than the RDs of monoculture-type and naïve plants. In contrast, the RD of leaf thickness was positively correlated with NEs and CEs for both monoculture and mixture types, but not for naïve plants (Fig. 4c). At the second harvest, NEs and SEs were significantly negatively correlated with the RD of plant height (Fig. 5). CEs were not influenced by interspecific variation in plant height, again not supporting hypothesis 6. SLA and leaf thickness were not measured at the second harvest.

Next, we looked at the relationship of biodiversity effects with community-weighted trait means CWMs). Whereas CEs were negatively correlated with the CWM of SLA (Fig. 6b), the SE was positively correlated with SLA (Fig. 6b, right panel). Consequently, NEs, driven by CEs, decreased with increasing SLA. Leaf thickness had a marginally significant effect on SEs, but the directionality depended on selection history. Plant height did not have a significant effect on any of the biodiversity effects at the first harvest. However, the interaction between trait means and selection history was significant for the relationship between the CWM of plant height and the SE at the first harvest. Thus, even though the trait mean did not have a direct impact on biodiversity effects, selection history influenced the trait means, which in turn influenced biodiversity effects. Selection history did not significantly impact the relationship between biodiversity effects and CWMs for the other

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two traits. At the second harvest, CWM of plant height had a significantly positive effect on NE, CE and SE (Fig. 7), hence the biodiversity effects were stronger for overall taller plants. However, when compared to the first harvest, the effect of selection history on the relationship between the CWM of plant height and the SE disappeared at the second harvest (Fig. 7). Overall, these results provided mixed evidence for hypothesis 7, which predicted a positive relationship between SEs and CWMs but no relationship between CEs and CWMs.

DISCUSSION

Influence of plant selection history on community productivity (hypotheses 1 and 4)

Previous research has shown that plant community productivity can be influenced by plant selection history, especially by the selection for increased niche differentiation in plants that had been grown for eight years in mixtures (mixture-type plants) compared to plants that had been grown in monoculture (monoculture-type plants, Zuppinger-Dingley et al. 2014). The present study included naïve plants without selection history in a biodiversity experiment. For plants with a selection history in the Jena biodiversity experiment (Roscher et al. 2004), we hypothesized that 2-species mixtures newly assembled with mixture-type plants should have greater community productivity than similar mixtures newly assembled with monoculture-type plants (hypothesis 1) and, conversely, that monocultures newly assembled with monoculture-type plants should have greater community productivity than similar monocultures newly assembled with mixture-type plants (hypothesis 4). For naïve plants, we expected intermediate community productivity in both monocultures and mixtures.

Our results provide mixed evidence for these hypotheses, in part depending on the particular species and species combinations. Thus, plant communities consisting of naïve plants without a selection history in the Jena Experiment often produced the lowest

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community biomass, especially in 2-species mixtures (see Fig. 1). It is conceivable that evolutionary processes in the field plots, where plants were grown for a longer time without re-sowing than was the case for the naïve plants in the propagation cultures of the commercial supplier, led to the increased performance of selected plants.

Comparing test communities consisting of either monoculture-type plants or mixture-type plants, we observed that mixture-type plants did have higher community productivity than monoculture-type plants in 2-species mixtures, as expected under hypothesis 1. But contrary to our expectation (hypothesis 4), mixture-type plants also produced more biomass than monoculture-type plants when grown in monoculture, thereby reducing biodiversity effects as discussed below. The generally lower performance of monoculture-type plants could have been due to selection for increased defense, trading off with reduced growth (Coley et al. 1985, Herms and Mattson 1992). The increased defense may not have become effective during the 24 weeks of growth in the present experiment. In a parallel glasshouse experiment with single individuals per pot, we indeed found greater pathogen damage on mixture- than on monoculture-type plants (Terhi Hahl et al., personal observation).

Within these main effects of selection history, we found large variation in selection-history effects among species in monocultures and among species compositions in 2-species mixtures. These findings emphasized the importance of conducting such studies with multiple species but at the same time sufficient replication for each in monoculture and for their combinations in mixture. High replication can more easily be achieved in experiments with one focal species (e.g. Kleynhans et al. 2016, Rottstock et al. 2017), but extrapolating results from such experiments might under- or overestimate overall effects of selection on the response of plants to different biotic conditions. In the present study, we used five focal species and already found strong differences regarding their selection response to community diversity.

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Influence of plant selection history on biodiversity effects (hypothesis 2)

Net biodiversity effects (NEs) can be partitioned into CEs and SEs. When CEs drive over-yielding, most species should contribute equally contribute to greater community productivity in mixtures, due to niche differentiation among them. Conversely, SEs are large when few dominant species are driving positive diversity–productivity relationships, because they benefit from growing in mixtures (Loreau and Hector 2001).

Naïve plants exhibited weak biodiversity effects, confirming findings from a field experiment (van Moorsel et al. 2017), where we found biodiversity effects to be weaker for communities assembled with naïve plants, especially when comparing monocultures with 2- and 4-species mixtures. As mentioned above, naïve plants in contrast to selected plants had not experienced the continued selection in field plots without re-sowing. Furthermore, they had not experienced interspecific competition before, which was at least the case for the monoculture types among the selected plants. Comparing the monoculture and mixture types, we found that at the first harvest NEs, CEs and SEs were larger for communities consisting of monoculture-type plants, which for the NEs and CEs was in contrast with our expectation (hypothesis 2). The lower CE for mixture-type plants was due to the good performance of mixture types in newly assembled monocultures and not because mixture types performed poorly in newly assembled mixtures. At the second harvest, NEs, CEs and SEs were rather similar for the two selection histories, thus no longer contradicting expectations, but also not supporting them (hypothesis 2). Nevertheless, at least in four 2-species combinations — *Lathyrus* + *Veronica*, *Galium* + *Veronica*, *Veronica* + *Prunella* and *Plantago* + *Prunella* — the directionality changed from the unexpected to the expected result, i.e. CEs at the second harvest were larger for mixture- than monoculture-type plants (see Fig. 2b). Over longer timespans, CEs often increase and SEs often decrease (van

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Ruijven and Berendse 2005, Fargione et al. 2007, Montès et al. 2008, Isbell et al. 2009, Marquard et al. 2009). It is conceivable that this would also have occurred in our experiment if it had continued beyond the 24-weeks timespan.

Influence of plant selection history on trait variation (hypotheses 3 and 5)

Because community-level trait variation can reflect niche differentiation (Violle et al. 2012, Roscher et al. 2015), we measured intra- and interspecific trait variation among individual plants in all communities. We hypothesized that mixture-type plants should exhibit larger trait variation between species as they underwent selection for increased complementarity during twelve years in the experimental field plots (hypothesis 3). Conversely, we expected stronger within-species trait variation in monoculture-type plants, due to 12 years of strong intraspecific competition in the experimental field plots (hypothesis 5). Overall we found that variation tended to be larger both within and between species for monoculture-type plants (see Fig. 3), thus not confirming hypothesis 3, but weakly confirming hypothesis 5. Several studies have investigated the relationship between species richness and community-level trait variation (Hulshof et al. 2013, Le Bagousse-Pinguet et al. 2014, Lamanna et al. 2014, Siefert et al. 2015) and found that the relative extent of intraspecific trait variation depended on species richness. In monocultures, a large intraspecific variation is advantageous for a more efficient use of resources, leading to our hypothesis 5. Thus, the observed trend for increased trait variation in monoculture types (see Fig. 3) is consistent with potential selection for within-species niche differentiation and character displacement in monocultures.

The lack of increased between species trait differences in mixture- compared with monoculture-type plants was in accordance with a lack of increased CEs for mixture-type plants. This contrasts with the results of an earlier study in which increased CEs of mixture-

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type plants were associated with increased between-species trait differences (Zuppinger et al. 2014). A potential explanation for the different results is that the earlier study used species which were more different among each other, namely grasses, legumes, small herbs and tall herbs, whereas species in the present study were more similar and therefore perhaps less likely to further increase their differences by short-term evolution than species which were more different to begin with. The species in the present study may have evolved "parallel" character displacement in response to species of the other functional groups also present in the mixtures in which they were selected in the Jena Experiment.

Influence of trait variation and community-weighted means on biodiversity effects (hypotheses 6 and 7)

One potential underlying mechanism for increased biodiversity effects observed in field experiments (Cardinale et al. 2007, Reich et al. 2012), could be selection for niche differentiation (Zuppinger-Dingley et al. 2014). Not all trait variation, however, corresponds to niche differentiation (Turcotte and Levine 2016). In particular, traits related to light availability may behave differently because of the asymmetric nature of competition for light, i.e. being tall is generally better than being small. Thus, variation in plant height could be expected to decrease when species are grown in mixtures rather than monocultures (Vermeulen et al. 2008, Roscher et al. 2015). Given the absence of increased CEs and between-species trait variation in mixture-type plants, the relationship between functional traits in our 2-species mixtures and biodiversity effects should not have differed according to plant selection history. Nevertheless, we could still test how trait variation and means were correlated with biodiversity effects. Specifically, we predicted that relative trait differences

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(RDs) should be positively related to CEs (hypothesis 6) and community-weighted trait means (CWMs) should be positively related to SEs (hypothesis 7).

In opposition to hypothesis 6, RDs in plant height were negatively rather than positively correlated with CEs and consequently NEs (see Fig. 4a, 5). This discrepancy of observation and expectation suggests that RDs in plant height may reflect competitive hierarchies rather than complementary of plants with respect to light use, as discussed above with regard to the asymmetry of light competition. At the second harvest, CWMs of plant height had a positive impact on all biodiversity effects (Fig. 7), i.e. not only on SEs — which we had expected under hypothesis 7 —, but in accordance with findings of previous studies (Vermeulen et al. 2008, Roscher et al. 2015).

Functional diversity in SLA within a community should increase complementary light use (Roscher et al. 2011). Leaf thickness is inherently related to SLA (White and Montes-R 2005) and might act similarly to SLA. In our study, RDs in leaf thickness, but not RDs in SLA, were positively correlated with all biodiversity effects, especially for mixture-type plants (see Fig. 4c). Hence, trait plasticity in leaf thickness was advantageous for species growing in mixtures. However, SEs was as much increased as CEs, whereas according to our expectation (hypothesis 6) positive correlations between trait differences should mainly involve CEs. Additionally, CWMs of SLA did have a positive effect on SEs, consistent with hypotheses 7, but also a negative effect on CEs, adding up to a negative effect on NEs (see Fig. 6b), suggesting that overall a smaller leaf area per unit mass for species growing in mixtures has a positive effect on productivity.

CONCLUSIONS

Here, we demonstrated that community diversity had the selective potential to alter species performances, which may in part explain the strengthening biodiversity–ecosystem

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functioning relationship observed in the field experiments (e.g. Reich et al. (2012)). Selection in a biodiversity experiment increased community productivity in newly assembled test communities compared to communities consisting of naïve plants without such selection history. Moreover, selection in mixtures increased community productivity in newly assembled mixtures and monocultures compared with selection in monocultures. These findings imply that co-evolutionary processes occurred throughout the 12-year selection period in the experimental plots of the biodiversity experiment and involving at least two sexual reproduction cycles.

Selection experiments like the present one should include a number of species and species compositions, because these may show different evolutionary responses, as observed in the present study. Studies with one focal species might either under- or overestimate the effects of "biodiversity selection" on the response to current assay conditions. Revealing such rapid evolutionary processes in grassland plant communities also has implications for conservation strategies. Thus, it may not be sufficient to only conserve species in isolation but rather in communities or populations of species with co-evolved interactions.

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experiment and S.J. van Moorsel, M.W. Schmid and B. Schmid analyzed the data. S.J. van Moorsel and B. Schmid wrote the manuscript with all other authors contributing to revisions.

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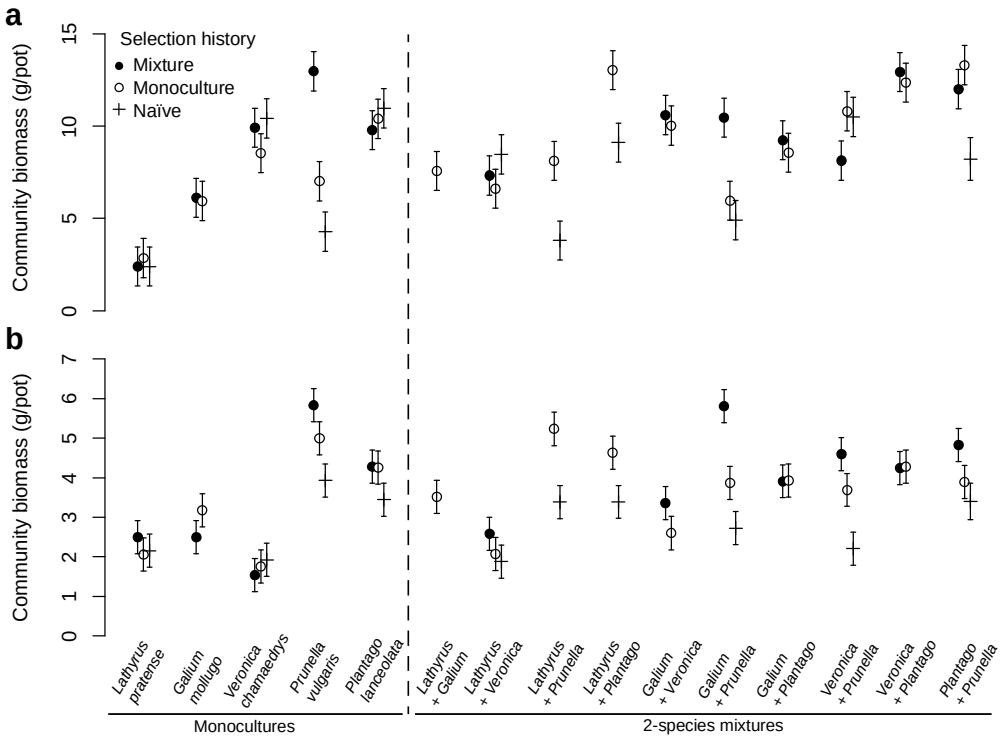

FIG. 1. Mean community biomass for monocultures and 2-species mixtures. Shown are means and standard errors from a linear mixed-effects model with selection history, species combination and the interaction between selection history and species assembly as fixed-effects terms and table (including the block) as random-effects term. **a**, first harvest. **b**, second harvest.

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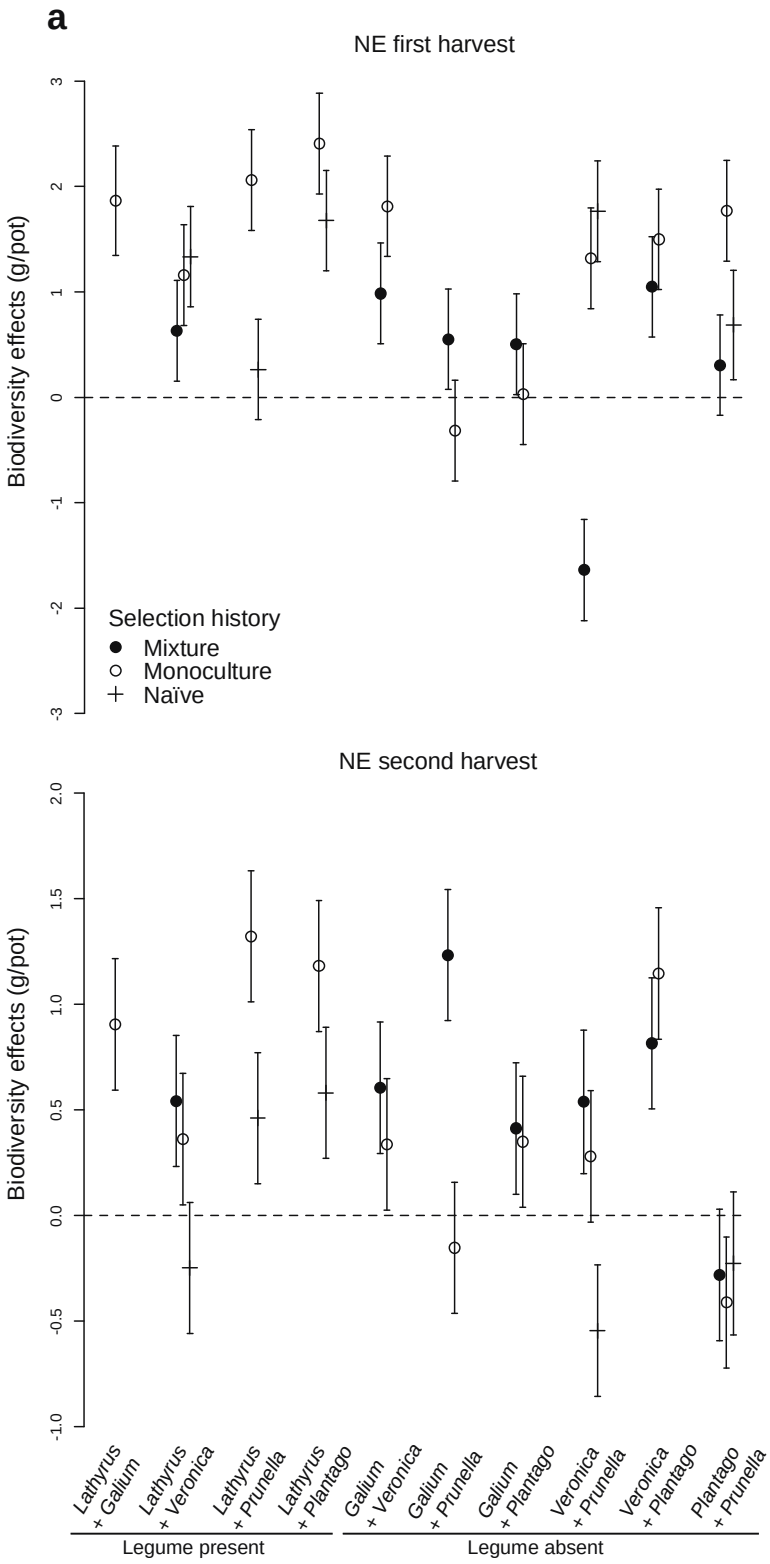

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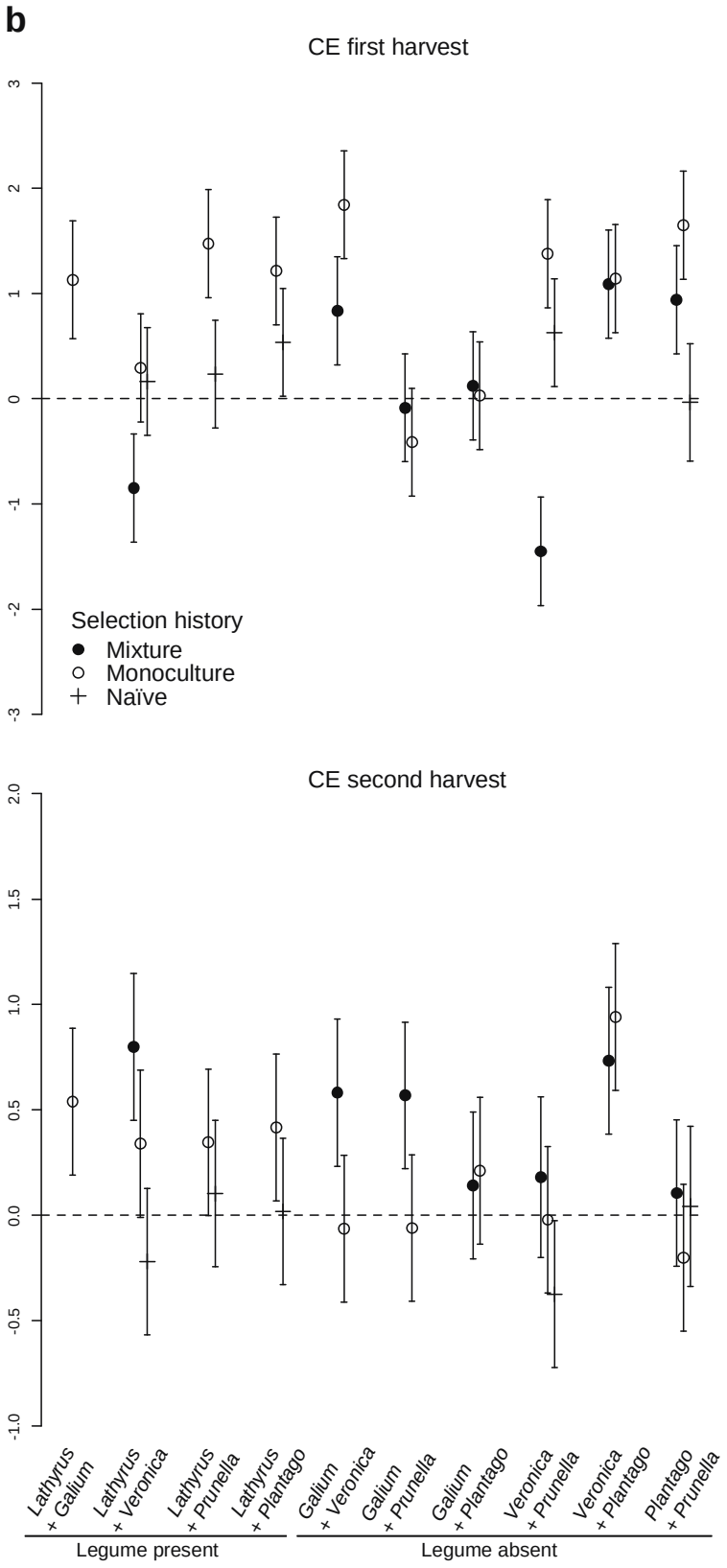

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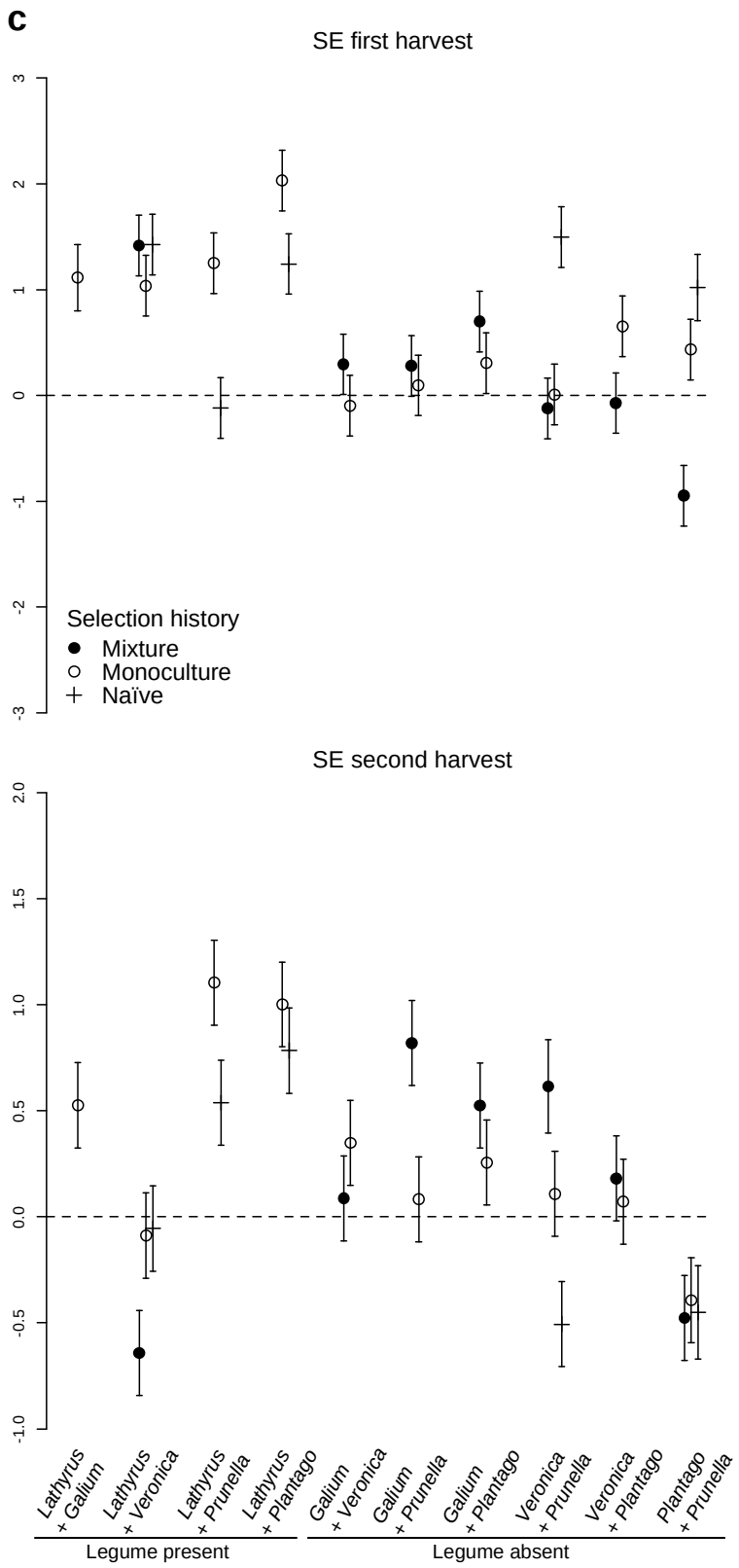

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FIG. 2. Biodiversity effects were assessed for both biomass harvests by additive partitioning of the net effect (**a,** NE) into complementarity effect (**b,** CE) and sampling effect (**c,** SE) for plants with different selection histories (naïve, monoculture, mixture). Shown are means and standard errors from a linear mixed-effects model, with selection history, species assembly and the interaction between selection history and species assembly as fixed-effects terms and table (including block) as random-effects term.

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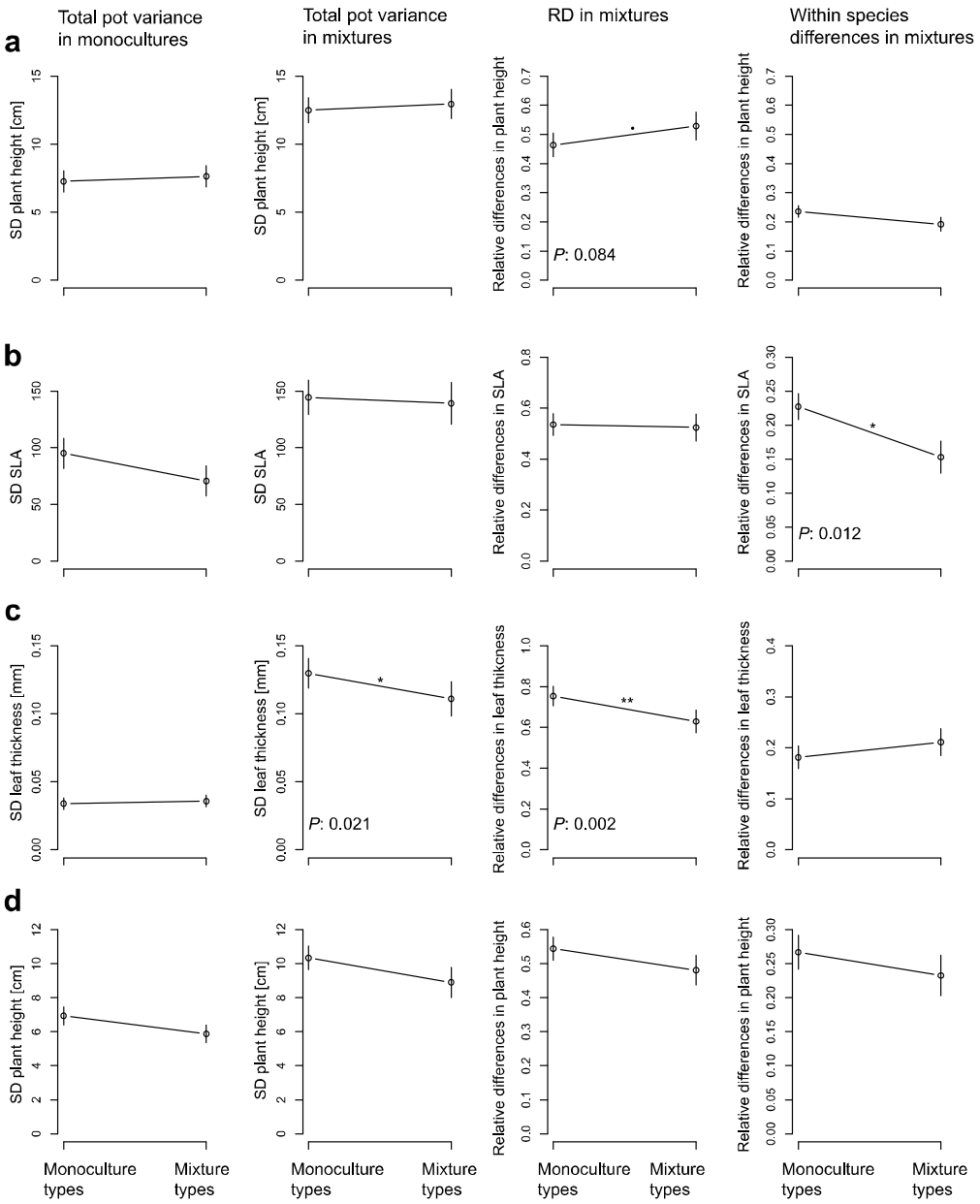

FIG. 3. Trait variance in monoculture and mixture assemblies in response to selection history (monoculture- vs. mixture-type plants). **a)** plant height at the first harvest, **b)** SLA at the first harvest, **c)** leaf thickness at the first harvest, **d)** plant height at the second harvest. Shown are means and standard errors from a mixed-effects model with selection history, species assembly and the two-way interaction of these as fixed-effects terms and table (including block) as random term. Significant and marginally significant *P*-values are indicated in the respective plot.

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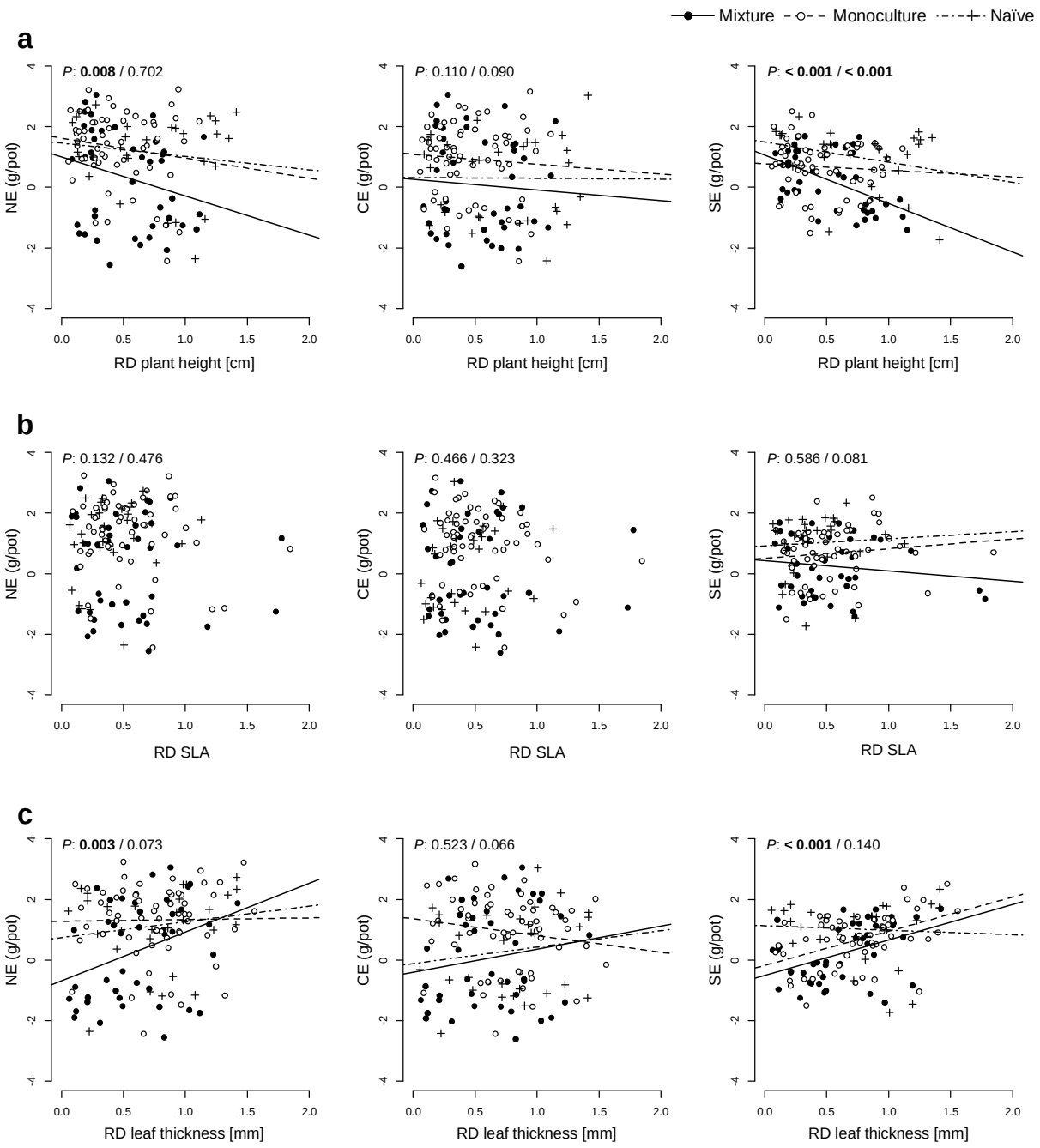

FIG. 4. Biodiversity effects at the first harvest in response to relative differences between species (RDs) for three traits: **a,** plant height (in cm), **b,** specific leaf area (SLA) and **c,** leaf thickness (in mm). Indicated *P*-values refer to ANOVA results for fixed-effects terms from a mixed-effects model with RD, species assembly, selection history and interactions of these

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as fixed-effects terms and table (including block) as random-effects term: RD / interaction RD × selection history (naïve plants vs. mixture types vs. monoculture types). Regression lines are plotted in cases for which at least one *P*-value was significant. Left column: NE, middle column: CE, right column: SE.

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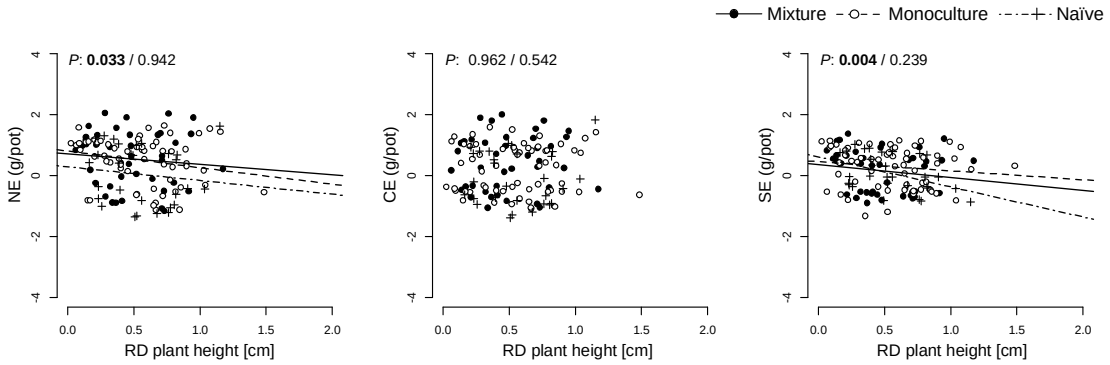

FIG. 5. Biodiversity effects at the second harvest in response to relative differences between species for plant height (in cm). Indicated *P*-values refer to ANOVA results for fixed-effects terms from a mixed-effects model with RD, species assembly, selection history and interactions of these as fixed-effects terms and table (including block) as random-effects term: RD / interaction RD × selection history (naïve plants vs. mixture types vs. monoculture types). Regression lines are plotted in cases for which at least one *P*-value was significant. Left column: NE, middle column: CE, right column: SE.

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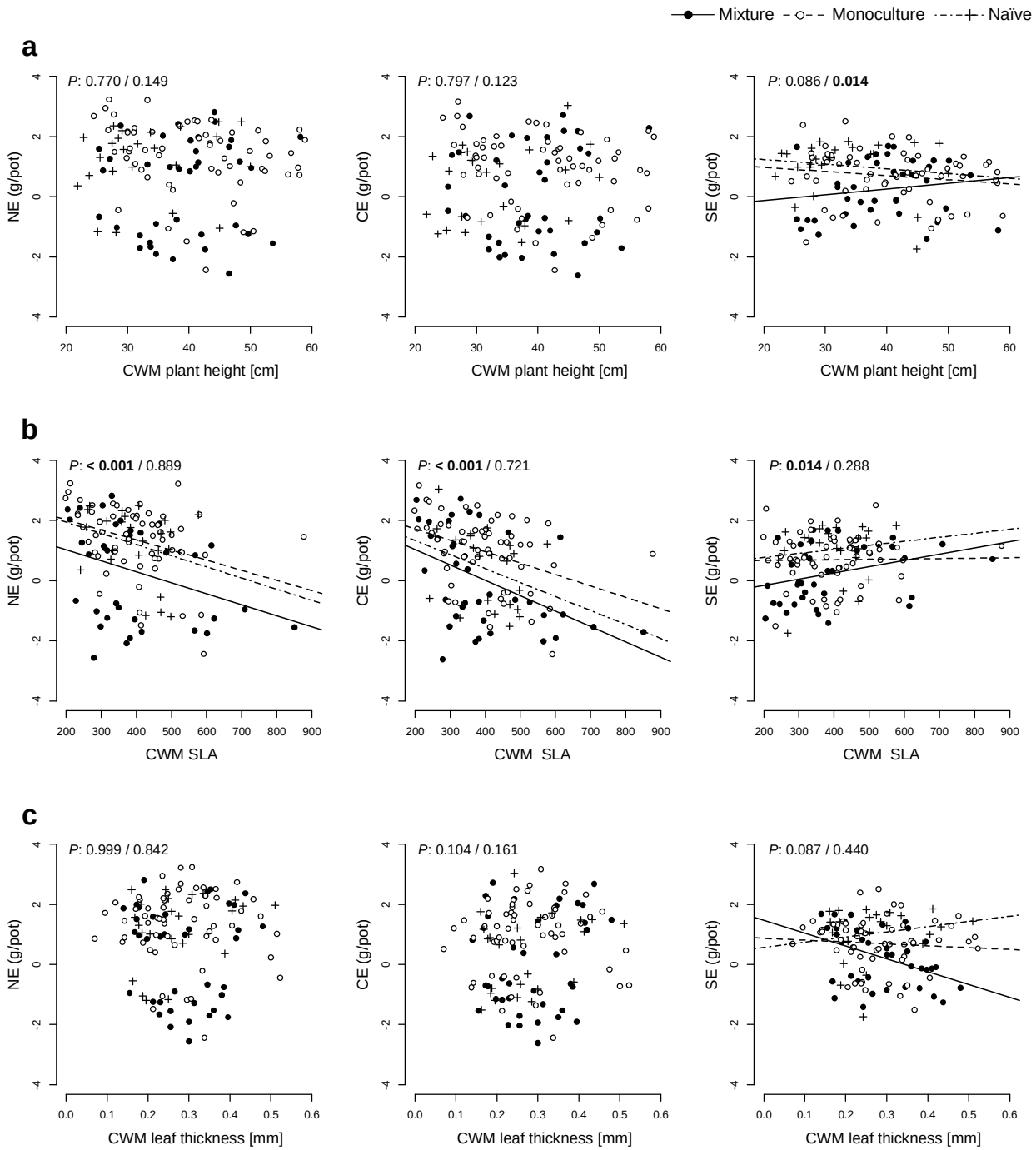

FIG. 6. Biodiversity effects at the first harvest in response to the community-weighted mean (CWM) of three traits: **a,** plant height (in cm), **b,** specific leaf area (SLA) and **c,** leaf thickness (in mm). Indicated *P*-values refer to ANOVA results for fixed-effects terms from a mixed-effects model with CWM, species assembly, selection history and interactions of these as fixed-effects terms and table (including block) as random-effects term: CWM /

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interaction CWM × selection history (naïve plants vs. mixture types vs. monoculture types). Regression lines are plotted in cases for which at least one *P*-value was significant. Left column: NE, middle column: CE, right column: SE.

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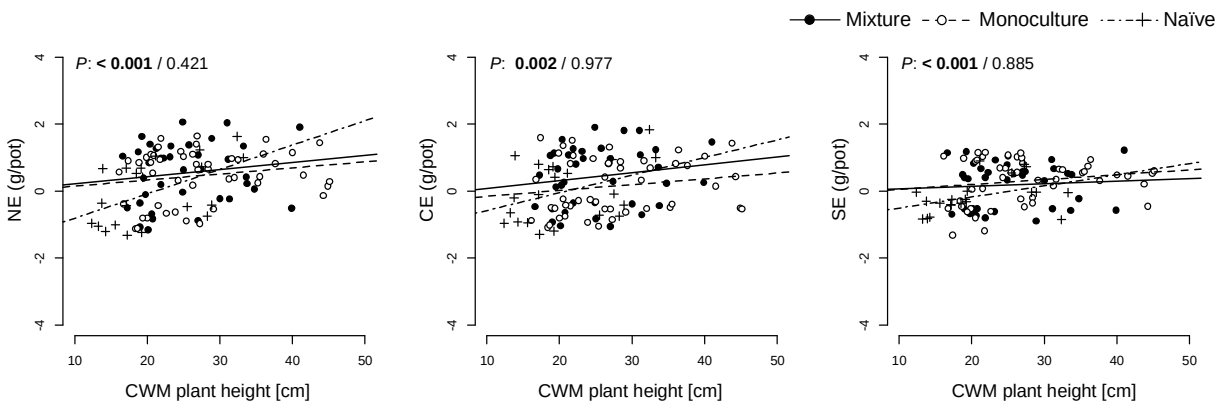

FIG. 7. Biodiversity effects at the second harvest in response to the community-weighted mean (CWM) of plant height (in cm). Indicated *P*-values refer to ANOVA results for fixed-effects terms from a mixed-effects model with CWM, species assembly, selection history and interactions of these as fixed-effects terms and table (including block) as random-effects term: CWM / interaction CWM × selection history (naïve plants vs. mixture types vs. monoculture types). Regression lines are plotted in cases for which at least one *P*-value was significant. Left column: NE, middle column: CE, right column: SE.

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TABLE 1. Summary of hypotheses.

Hypothesis	
1)	Mixture-type plants produce high biomass in mixtures.
2)	Mixture-type plants have large NEs and CEs.
3)	Mixture-type plants show large interspecific trait variation.
4)	Monoculture-type plants produce high biomass in monocultures.
5)	Monoculture-type plants show large intraspecific trait variation.
6)	Large CEs are due to between-species trait variation.
7)	Large SEs are due to large CWMs.

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TABLE 2. Results of mixed-effects ANOVA for the aboveground biomass of the test communities.

		Harvest 1			
Source of variation		**nDf**	**dDF**	***F***	***P***
Species assembly:					
	Monoculture vs. mixture	1	173.3	29.09	< 0.001
	Monoculture identity or species combination of mixture	13	171.2	16.53	< 0.001
Selection history:					
	Naïve vs. mono or mix types	1	173	16.63	< 0.001
	Mono vs. mix types	1	169.6	1.78	0.184
Assembly × history:					
	Monoculture vs. mixture × naïve vs. mono or mix types	1	168.4	1.72	0.191
	Monoculture vs. mixture × Mono or mix types	1	172.2	1.69	0.195
	Species assembly × naïve vs. mono or mix types	8	171.7	5.35	< 0.001
	Species assembly × mono types vs. mix types	10	172.3	2.91	0.002
Variance components		**n**	**Var**	**SE**	
	Table (including blocks)	18	1.7512	0.8010	
	Residual (pots)	221	5.8403	0.6395	

		Harvest 2			
Source of variation		**nDf**	**dDF**	***F***	***P***
Species assembly:					
	Monoculture vs. mixture	1	174	10.78	0.001
	Monoculture identity or species combination of mixture	13	171.8	15.47	< 0.001
Selection history:					
	Naïve vs. mono or mix types	1	173.7	42.72	< 0.001
	Mono vs. mix types	1	170.1	5.71	0.018
Assembly × history:					
	Monoculture vs. mixture × naïve vs. mono or mix types	1	168.8	8.56	0.004
	Monoculture vs. mixture × Mono or mix types	1	172.9	3.52	0.062
	Species assembly × naïve vs. mono or mix types	8	172.3	2.15	0.033
	Species assembly × mono types vs. mix types	10	172.9	1.23	0.275
Variance components		**n**	**Var**	**SE**	
	Table (including blocks)	18	0.2451	0.1145	
	Residual (pots)	221	0.9225	0.1009	

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Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, *F* = variance ratio, *P* = probability of type-I error. Variance components (Var) and associated standard errors (SE) for the random effects are provided together with the number of replicates.