A plant biodiversity effect resolved to a single genetic locus

Samuel E. Wuest[1,2,*] and Pascal A. Niklaus[2]

1) Department of Plant and Microbial Biology and Zurich-Basel Plant Science Center, University of Zurich, Zollikerstrasse 107, 8008 Zurich

2) Department of Evolutionary Biology and Environmental Studies and Zurich-Basel Plant Science Center, University of Zurich, Winterthurerstrasse 190, 8057 Zurich

*correspondence to: Samuel E. Wuest (samuel.wuest@ieu.uzh.ch)

Summary

There is now pervasive evidence of positive effects of biodiversity on plant community productivity and functioning[1,2]. Although some advances have been made linking diversity effects to functional trait variation[3], progress towards a mechanistic understanding remains slow[4] - in part because biodiversity effects are emergent complex properties of communities, and mechanisms might differ between communities or environmental conditions[5]. Without a mechanistic understanding, however, the advancement of ecological theory as well as applications in agriculture are impeded. Here, we analyse non-additive interactions between divergent Arabidopsis accessions in experimental plant communities. By combining concepts and designs from ecology and plant breeding with genetic methods, we have identified a major effect locus at which allelic diversity promotes community productivity. In further experiments with near-isogenic lines, this diversity effect locus was resolved to a single region representing less than 0.3% of the genome. Using plant-soil-feedback experiments, we demonstrate that allelic diversity causes genotype-specific soil legacy responses in a subsequent plant generation. This suggests that asymmetric interactions of plants with soil-borne factors drive niche complementarity and that the impacts of allelic diversity can extend across generations. In summary, this work shows that positive diversity effects can be linked to single Mendelian factors, and that complex community properties can have simple causes. This may pave the way to novel breeding strategies, which focus on phenotypic properties that do not manifest themselves at the individual level, but only at a higher level of biological organisation.

Main Text

More than two decades of plant ecological research and the publication of hundreds of studies have firmly established that positive biodiversity effects, in particular on community yield, are the rule rather than exception and are often substantial[1,2]. These effects have been explained by larger community-level resource use promoted by niche complementarity, and by reduced negative

density-dependent effects of enemies[6,7]. Yet, our understanding of the particular driving mechanisms remains poor, for several reasons. First, diversity effects are emergent properties that only manifest in comparisons of communities differing in diversity[8]. Second, diversity effects, and the mechanisms that drive these, may change with environmental conditions[5,9]. Third, while there is no doubt that functional trait differences underly biodiversity effects[10], trait-based analyses remain to some degree phenomenological because evolutionary forces have led to the formation of trait syndromes, i.e. to sets of highly correlated traits that reflect fundamental trade-offs between ecological strategies[11,12]. The observed variation in traits thus is confounded with phylogeny[13], and it remains almost impossible to distinguish the traits that are true drivers of biodiversity effects from traits that are merely correlated. Our still poor mechanistic understanding of biodiversity effects impedes the development of predictive ecological theory and their implementation in, for example, agricultural applications.

Most biodiversity research to date has focused on variation among species, but experimental[14], theoretical[15], and observational[16] studies have shown that positive diversity effects on productivity also occur at levels of organization above and below species. An important part of the trait variation apparent in plant communities occurs within species[17], and increased intra-specific variation can have similar effects as inter-specific trait variation in low-diversity systems[14,18,19]. Despite qualitative differences, there may therefore be commonalities of trait variation within and between species with respect to effects and mechanisms, indicating that studies at the genotype level may provide some insights into effects of species-level variation and vice versa. A methodological advantage of intra-specific biodiversity studies is that genetic methods can circumvent some of the problems encountered in species-level diversity studies. Specifically, crosses between genotypes allow trait variation between individuals to be re-arranged[20] without confounding with population

structure or the differentiation into ecological strategies. However, genetic approaches are normally used to study properties of individuals rather than emergent properties of communities.

Here, we demonstrate in a case study how the genetic approach can be harnessed to identify the genetic underpinnings of biodiversity effects. By screening pair-wise mixtures of divergent natural accessions of *Arabidopsis thaliana*, we identified two accessions, Bayreuth (Bay) and Shadhara (Sha), that exhibit positive net biodiversity effects when grown together, i.e. mixtures produce a higher community-level biomass than the average of their monocultures (Fig. 1a). This effect depended on soil conditions, with effects that were essentially absent on peat-rich soil but grew to an overyielding of 16% with increasing amounts of sand in the substrate (sand content × diversity: $F_{1,160}$= 4.57, $P < 0.05$; Fig. 1b and Extended Data Fig. S1a). Analysis by additive partitioning[8] revealed that these community-level biodiversity effects were due to complementarity rather than selection effects (sand content × complementarity effect, $F_{1,77} = 7.21$, $P < 0.01$; Extended Data Fig. S1b); specifically, this indicates that both ecotypes benefited from growing in mixed communities.

To analyse the genetic basis of the positive diversity effect in mixed Bay-Sha communities, we performed quantitative trait locus (QTL) mapping using publicly available recombinant inbred lines (RILs). These RILs are largely homozygous (Fig. 1c) and have been derived from a cross between the Bay and Sha accessions, followed by multiple subsequent rounds of inbreeding[21]. For efficient mapping, we capitalized on a so called competition diallel. Traditional diallel designs systematically cross parental lines and are used in breeding to determine the genetic basis of traits; specifically, diallel analysis partitions the traits of crosses into additive contributions[22] of parental lines (general combining abilities; GCA) and cross-specific effects (specific combining abilities; SCA), with the latter interpreted as consequences of dominance or epistasis. By substituting individuals and crosses

with communities and mixtures, the principle of diallels can be applied to the analyses of biodiversity effects in communities[23], which we did here (Fig. 1c, d). In this context, the distinction between maternal and paternal effects ceases to apply, simplifying the design to a half-diallel. SCAs then quantify the deviations of mixture yields from expectations based on additive average contributions of the two genotypes. We combined 18 RILs and the two parental accessions Bay and Sha, in four replicate blocks, on sand-rich soil. We detected significant positive genotype diversity effects (Fig. 2a, $F_{1,189}$= 10.47, P<0.01), indicating that the traits that promote biodiversity effects are heritable. As expected, a large proportion of the variation in SCA remained unexplained. We therefore tested for allelic diversity effects on SCAs at 69 marker positions. Both a marker regression technique and a standard QTL procedure revealed a major effect locus on the lower arm of chromosome four where allelic diversity at the community level resulted in higher SCAs (Fig. 2b and Extended Data Fig. S2).

With 18 recombinant lines, mapping resolution was limited and other effect loci or genetic interactions among loci may have gone unnoticed. We thus aimed at resolving the allelic diversity effect further to a single Mendelian factor. For this, we isolated a family of 19 near isogenic lines (NILs) that genetically varied only on the lower arm of chromosome 4, and in which we selected and inferred further recombination events by molecular markers and whole-genome re-sequencing (Fig. 3a,b, Extended Data Fig. S3). With these NILs, we performed a second diallel experiment, replicated once on peat-rich soil where we expected no diversity effects and once on sand-rich soil where we expected positive diversity effects. Indeed, no locus was associated with positive allelic diversity effects on peat-rich soil (Fig. 3c). In contrast, we found a positive allelic diversity effects at a single locus (overyielding of 4.5%, Fig. 3d, P < 0.01), represented by a region of approx. 310 kb in size (termed locus Chr4@16.92: between 16.92 to 17.23 Mb). Overall, two subsequent competition diallel experiments using only 37 recombinant lines were sufficient to resolve a plant

biodiversity effect to a genomic region representing ~2.5‰ of the Arabidopsis genome (containing approx. 86 genes), which emphasizes the extreme efficiency of our approach. The overyielding of allelic mixtures of otherwise isogenic lines was transgressive, i.e. allelic mixtures in the NIL diallel produced more biomass than the best mono-allelic communities ($t = 2.32$; $P = 0.02$). Transgressive overyielding is generally accepted as evidence of diversity effects that are driven by niche complementarity. Our work thus suggests that niche complementarity between genotypes can ultimately be attributed to diversity at discrete hereditary units.

Interactions between plant and soil factors have been invoked to explain productivity responses in plant biodiversity experiments[6,7,24], and the increase in biodiversity effects with time[25]. To test for soil-borne effects of allelic diversity on the growth of subsequent generations of plants, we performed soil feedback experiments with soil from both diallels, i.e. we grew phytometers[26] on soil pre-conditioned ("trained") by the growth of the specific genotype combinations (Fig. 1d and Supplementary Discussion). These phytometers were the two parental accessions Bay or Sha for the RIL diallel, and two near-isogenic lines in the NIL diallel. We found phytometer-specific soil legacy responses that depended on the allelic diversity of the communities that had conditioned the soils (Fig. 4; RIL diallel: diversity at marker MSAT4.9 × phytometer; $F_{1,166} = 6.48$; $P = 0.012$; NIL diallel: diversity at locus Chr4@16.92 × phytometer; $F_{1,168} = 5.61$; $P = 0.02$; Extended Data Table S1; Extended Data Fig. S4). Importantly, these phytometer-specific responses to soil legacy were independent of differences in previous community productivity and associated resource depletion (the effects remained statistically significant and comparable in size when first adjusting for community biomass in linear models). Interestingly, legacy effects of plants bearing the Bay allele differed in the experiments with RILs and NILs. This indicates that genotypes with the Bay allele in both cases strongly conditioned soils and drove subsequent legacy effects. However, and not unexpectedly given the complexity of mechanisms involved, these allele-specific effects cannot be

understood in isolation but depend on environmental conditions or genetic context (Supplementary Discussion). Developing a full understanding of the biological mechanisms at play will thus require further experiments, including soil analyses. Yet, our experiments demonstrate that plant genotype interactions mediated through allelic diversity at a single locus extend their effects across generations through their soil legacy. We were intrigued to find that we could in principle have genetically mapped this allelic diversity effect solely through its soil legacy; in other words, by QTL mapping an extended phenotypic property of allelic mixtures (Extended Data Fig. S4e,f; Supplementary Discussion).

Our study is, to the best of our knowledge, the first to systematically resolve biodiversity effects to their genetic origin. So far, complex emergent properties of plant communities did not necessarily seem genetically tractable, especially since quantitative traits of individuals often are polygenic[27] if not omnigenic in nature[28]. A single case study obviously is limited with respect to generalizations, but we consider it possible that in many cases complementarity and resulting biodiversity effects might instead have a relatively simple genetic architecture – a feature not uncommon for other types of biotic interactions[29]. Our genetic approach is extensible to the study of interactions among other genotype combinations and, with modifications, among species, and could thus lead to fundamental new insights into the traits and genetic underpinnings of biodiversity effects in more natural systems. Equally importantly, the genetic tractability of such effects may allow efficient breeding of genotype mixtures that support increased yields through niche complementarity while maintaining low variation in economically relevant traits. Biodiversity effects have received relatively little attention in breeding and conventional agriculture, with the notable exception of crop rotation[30] and intercropping of cultivars and species[31–33]. Instead, sustaining a growing global human population heavily depends on increasing nutrient inputs to crop production systems[34], on breeding of single genotypes for monoculture performance[35], and on the use of within-individual diversity effects

termed heterosis[36]. Our approach might help bypass constraints imposed on the performance of single genotypes, by shifting breeding efforts from the individual to the system level[37].

Methods

Germplasm

The Shadhara and Bayreuth accessions were kindly provided by Nuno Pires (University of Zurich) and had originally been obtained from the Nottingham Arabidopsis stock center (NASC). The 18 RILs (representing the "RIL-minimal set" and line 33RV191 used to generate NILs (all contained in the core-pop set of 165 lines) were ordered from the Versailles Arabidopsis stock center (*http://publiclines.versailles.inra.fr*) and propagated in a growth chamber. A Bay×Sha RIL (33RV191) was confirmed to be heterozygous at two PCR marker positions on chromosome 4 (Table S2). Upon selfing of this line, the two NILs 33RV191-Sha and 33RV191-Bay were isolated, and their genomes were re-sequenced as described below. Furthermore, after selfing of another single heterozygous F_{10} individual of line 33RV191, we screened 160 offspring for recombination between the ShaBa5, ShaBa6 and ShaBa8 markers on chromosome four. Upon selfing of 23 putative recombinant offspring, we isolated 19 homozygous recombinant lines for which we confirmed a recombination event in the region by PCR. We then performed whole-genome re-sequencing to confirm the isogenic background and to infer recombination breakpoints for this heterogeneous inbred family (referred to as NILs throughout the text) as described below.

Soils and growth conditions

Soils consisted of different mixtures of a peat and nutrient rich soil (Einheitserde ED73; pH ~5.8, N 250 mg L^{-1}; P_2O_5 300 mg L^{-1}; 75% organic matter content; Gebrüder Patzer GmbH, Sinntal-Jossa, Germany) and finely grained quartz sand. Pot for all mixture experiments were 7×7×8 cm in size. The experiment using the parental lines Bay and Sha was replicated on a soil quality gradient with

sand contents of 0%, 40%, 75% and 80%, which resulted in a near-linear decrease of pot-productivity from the highest to lowest ED73 content (Fig. S1). For the rough-mapping of the diversity effect using RILs, we used a mixture of 80% sand and 20% ED73. For the fine-mapping diallel using NILs, we used either a 80%:20% or a 20%:80% sand:ED73 mixture.

Seeds were sown directly onto soils (approx. 10 seeds per position, 4 positions per pot, Fig. 1a). The pots were placed in growth chambers or greenhouse compartments and covered with plastic lids to maintain a high humidity for germination and initial seedling establishment. Additional light was provided if necessery, achieving a photoperiod of 14–16 hours. Day-time and night-time temperatures were maintained around 20–25 °C and 16–20 °C, respectively. Seedlings were thinned continuously until a single healthy seedling remained per position.

Once seedlings were established, the pots were placed in a greenhouse compartment with automated watering (every 2 days). In summer 2015, daytime temperatures were extremely high, and the first block of the RIL competition diallel was therefore grown in a growth chamber with full climate control (8 h night/16 h day; 60% humidity; 18/23°C night/day temperature). The second block was grown in the growth chamber for a month before it was re-located to the regular greenhouse compartment.

Pots that did not contain all four originally planted individuals were discarded. Plants were harvested 43–51 days after sowing, with the specific harvest date determined by the occurence of approx. 5–10 dehiscent siliques on the earliest flowering genotypes within a block.

After the competition diallels were harvested, soil feedback trials were established by dividing the soil of a pot into two smaller pots 5.5×5.5×6.0 cm in size. The respective phytometers (Bay or Sha for the RIL diallel, 33RV191-Sha or 33RV191-Bay for the NIL diallel) were sown directly onto the soil. Again, seeds were oversown and seedlings thinned continously until a single healthy individual remained. Phytometer experiments were harvested either 36 days after sowing (peat-rich soil remaining after the NIL diallel, harvested early because plant roots started to grow out of the pots)

or 49–58 days after sowing (sand-rich soil, each block was harvested on a single day). For all experiments, the position of the individual pots was randomized across trays during seedling establishment, and across watering tables after seedling establishment. Throughout the experiment, pots were re-positioned randomly within trays and tables every 7–10 days. Pots were watered *ad libitum*, and in case of high population densities of dark-winged fungus gnats, the systemic insectizide ActaraG (Syngenta Agro AG) was applied according to the manufacturers recommendation. After harvesting, plant biomass was dried at 65°C for at least three days before weighing.

Experimental designs

To test soil effects on biodiversity effects in mixed Shadhara-Bayreuth communities, four soil substrates varying in sand content were prepared as described above. We then grew 12 replicate monocultures of each accession plus 24 replicate mixtures per soil type (total of 48×4= 192 pots).

The RIL competition diallel consisted of a half diallel replicated in four blocks. All pair-wise RIL combinations were realized once per block except for RIL monocultures which were replicated twice. For the follow-up soil feedback experiment, we re-used soil from only the first two blocks of the competition diallel. We re-mixed the soil of each single pot after harvesting the plants, and re-distributed it into two smaller plots that were sown with either a Shadhara or Bayreuth parental genotype that served as phytometers.

The NIL competition diallel used for fine-mapping was realized in a single block that contained all pair-wise combinations of the 19 NILs including monocultures. The subsequent soil feedback stage was realized as described for the RIL diallel, using either 33RV191-Bay or 33RV191-Sha genotypes as phytometers.

Genotyping and line re-sequencing

PCR-based genotyping assays (Table S2) were developed based on deletions in the Sha genome as predicted by the Polymorph tool (http://polymorph.weigelworld.org)[38].

Barcoded libraries for genome re-sequencing were prepared using the Illumina Nextera DNA Library Prep Kit (FC-121-1031, Illumina Inc. San Diego, CA) in combination with the Nextera Index Kit (96 indices, FC-121-1012) and pair-end sequenced on an Illumina HiSeq 2500 (2x150 bp, rapid run). The clustering and sequencing were performed at the Functional Genomics Center Zurich. Sequences were aligned to the Arabidopsis genome (Col-0 genome, TAIR version 10) using BWA[39], aligned read sorting and variant calling were performed using samtools[40]. Aligned genomic sequences of the parental accessions Bay-0 and Sha were downloaded from the 1001 genomes project data center (http://1001genomes.org). The VCF-file produced by the samtools software was loaded into the R Statistical Software[41], where the subsequent analyses were performed: variant calls were filtered (for differences in genotype calls between the Sha and Bay genomes, quality of variant calls, population-level minimal minor allele frequency 0.2; maximum heterozygosity 0.2). Inference of genotype calls at polymorphic sites was performed as described previously[42] and inference of parental alleles was improved using functionality implemented in the MPR package[42]. Genotype reconstruction was then performed in R using a simple hidden Markov model as implemented in the R package HMM, with hidden state starting probabilities (Bay, Het or Sha) all set to 1/3, and transition probabilities from one state to itself set to 0.99998 and to the other two states set to 0.00001 each. Emission probabilities of genotype calls given a state, e.g. Bay, were set to 0.35, 0.25, 0.25, 0.15 for genotypes calls Bay, Het, Sha or missing, etc.

Statistical analyses

We analyzed data from the diallel experiments using linear mixed models summarized by analysis of variance (ANOVA). The model terms included, in this order, the general combining abilities (GCA) of genotypes (a factor with 20 levels in the RIL diallel and 19 levels in the NIL diallel), the

genotype diversity in the pot (GD, 1 or 2 genotypes), the allele identity in the genotype monocultures (A, Sha or Bay), the allelic diversity in the genotype mixtures (AD, 1 [Sha/Sha or Bay/Bay] or 2 [Sha/Bay]), and the genotype composition planted in the pot (comp). The factor GCA was created by superimposing the model matrices for factors coding for the first and second genotype (factors with 20 and 19 levels for RIL and NIL diallels, respectively). The significance of GD, A, and AD were determined using F-tests with comp as error term (denominator). A and AD were encoded in such a way that these contrasts applied only to genotype monocultures and mixtures, respectively. Technically, this was achieved by including a third level in the factor that did not vary in the other group. Fitting A and AD after GD therefore only explained variance in these subsets. The diallel model was extended by additional terms and the corresponding interactions when these applied; specifically, the RIL diallel included a block effect. The NIL diallel included terms for soil type, and interactions of all the terms above with soil type (for example, soil×AD was tested using soil×comp as error term). The soil feedback experiments included further interactions with phytometer (RIL and NIL diallel), and phytometer×soil (NIL diallel). Effects of pot biomass in the competition diallel (diallel biomass and diallel biomass × soil) were accounted for in these linear models, and data were square-root transformed to obtain normally distributed residuals.

Specific combining abilities for mapping were calculated directly, within blocks, by solving the linear model m = X GCA + SCA where X is the design matrix describing the genotype composition of a pot. Monoculture SCAs were also determined but not used for QTL mapping of allelic diversity within RIL mixtures. In the RIL diallel, the SCAs of each genotype composition was first calculated per block and then aggregated over all blocks using least-square estimates. Marker regression was performed contrasting SCAs of mono-allelic RIL mixtures (“BB” and “SS” compositions) with mixed-allelic mixtures (“BS” compositions) using the glht-function provided by the multcomp package[43]. QTL mapping was also performed using the R/qtl package and interval mapping (scanone-function), with both mono-allelic compositions at a given locus re-coded as to the same

level ("mono-allelic") and compared against mixed-allelic compositions. Genome-wide significance was assessed by resampling (n=5000).

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Author contributions

S.E.W. conceptualized and designed the research (with input from P.A.N.) and performed the experiments. Both authors performed the analyses and wrote the manuscript. Both authors read and approved the final version of the manuscript.

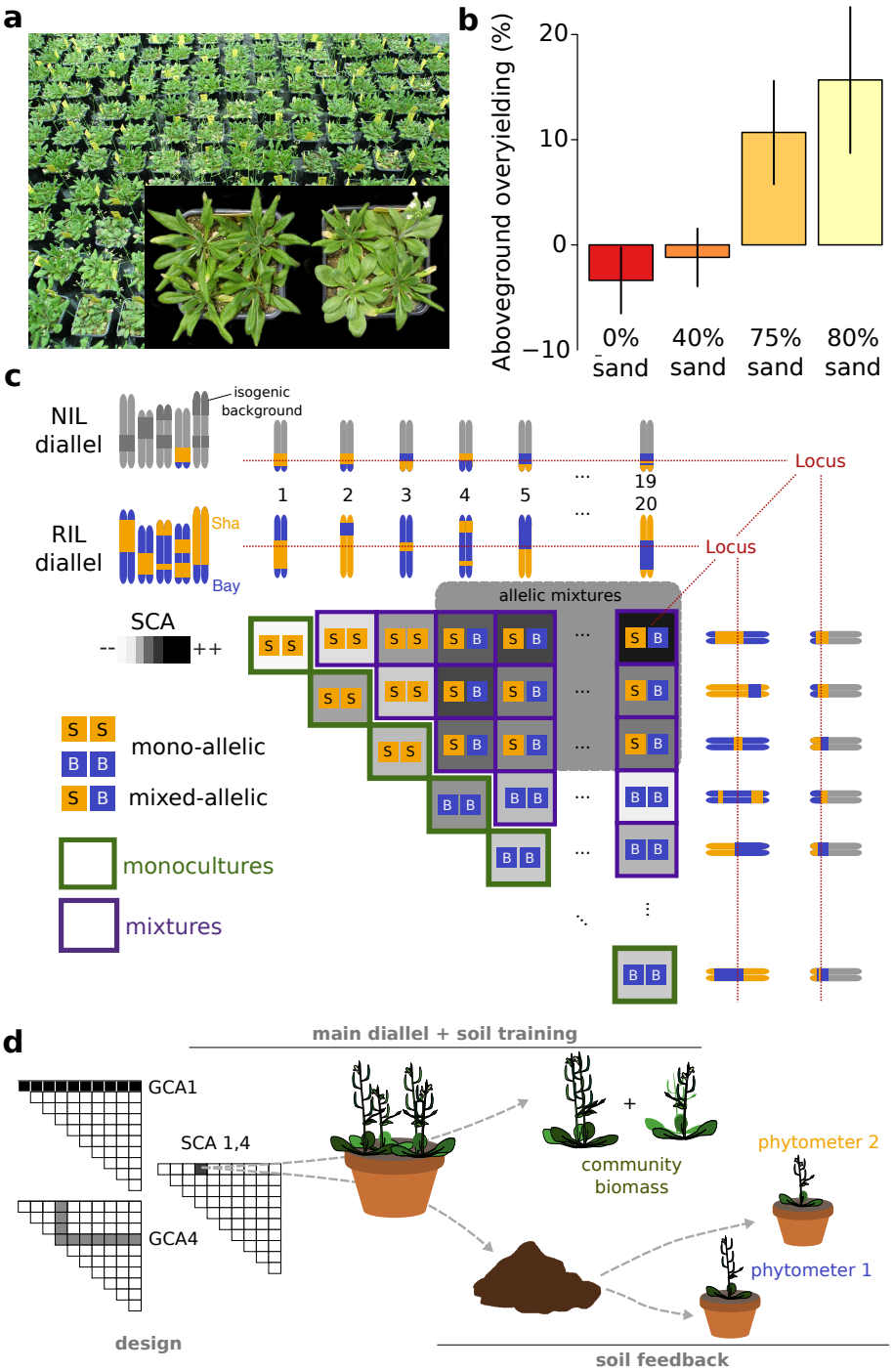
Data availability

The datasets described in the paper are available through the Zenodo data repository (DOI:XXX). Sequencing data are deposited in the NCBI Short-Read Archive (accession XXX).

Analysis scripts are available from the authors upon request.

Competing interests

The authors declare no competing financial interests.



398 **Figure 1 | Combining ecological concepts and genetic methods. a,** Pot systems used to study
399 diversity effects in pair-wise genotype mixtures. The inset shows a Recombinant Inbred Line (RIL)
400 monoculture (left) and mixture (right). **b,** Net diversity effects in Bay-Sha mixtures along a peat-
401 sand substrate gradient. Error bars denote standard errors of means (s.e.m.). **c,** Outline of the
402 competition diallel design and the genotypes used throughout this paper. 18 RILs and the two
403 parental accession, or 19 near-isogenic lines (NILs) were each placed in competition with each

other, allowing to assess i) effects of genotypic mixture (i.e. diagonal vs off-diagonal), or ii) effects of allelic mixture at a given locus across all genotype mixtures (i.e. comparing SS and BB vs. SB) on pot productivity. **d,** Outline of the experimental procedure used in this work. Colored labels indicate measured variables. GCA = general combining ability; SCA = specific combining abilities.

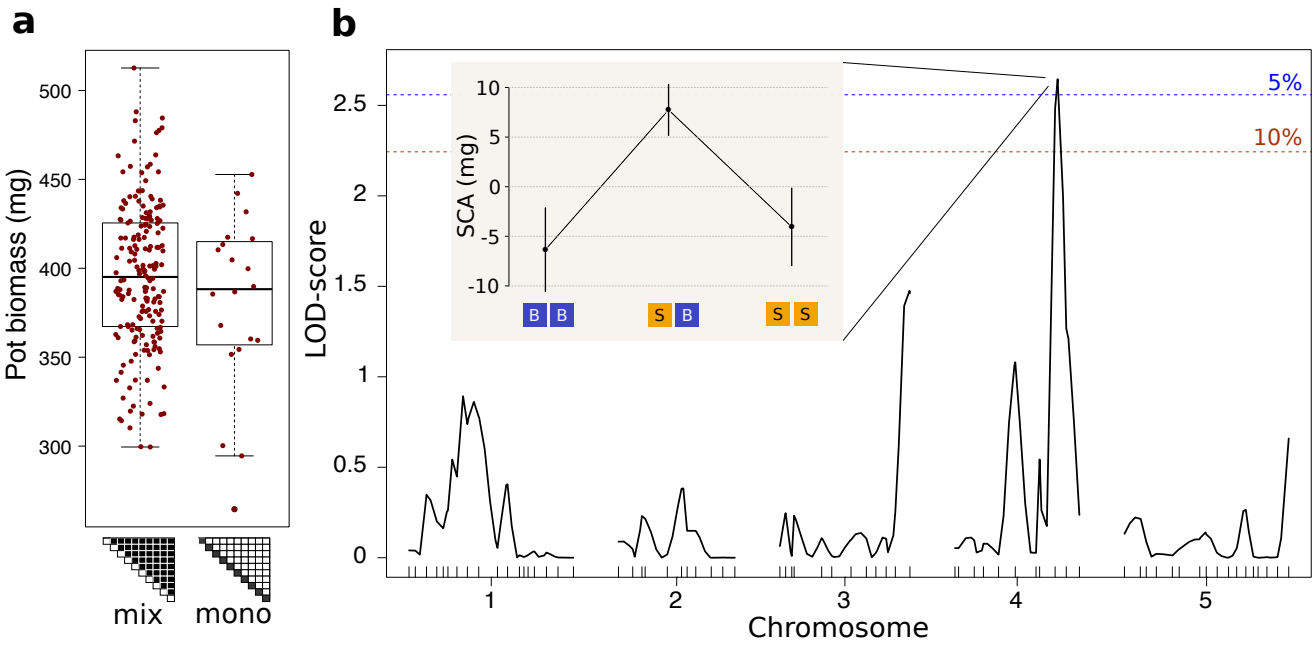


Figure 2 | Allelic diversity at a major effect locus increases community productivity. a, Pot-level productivity in dependence of community type (mix = RIL mixtures vs mono = RIL monocultures), showing positive genotype mixture effects in the diallel and on sand-rich soil (values aggregated across four blocks). **b,** Quantitative trait locus interval mapping of allelic diversity effects on specific combining ability (SCA). Vertical lines denote 10% and 5% genome-wide significance levels. The inset shows estimated SCA (± s.e.m.) across genotype mixtures that exhibit different allelic compositions at marker MSAT4.9 on chromosome four. LOD: logarithm of the odd.

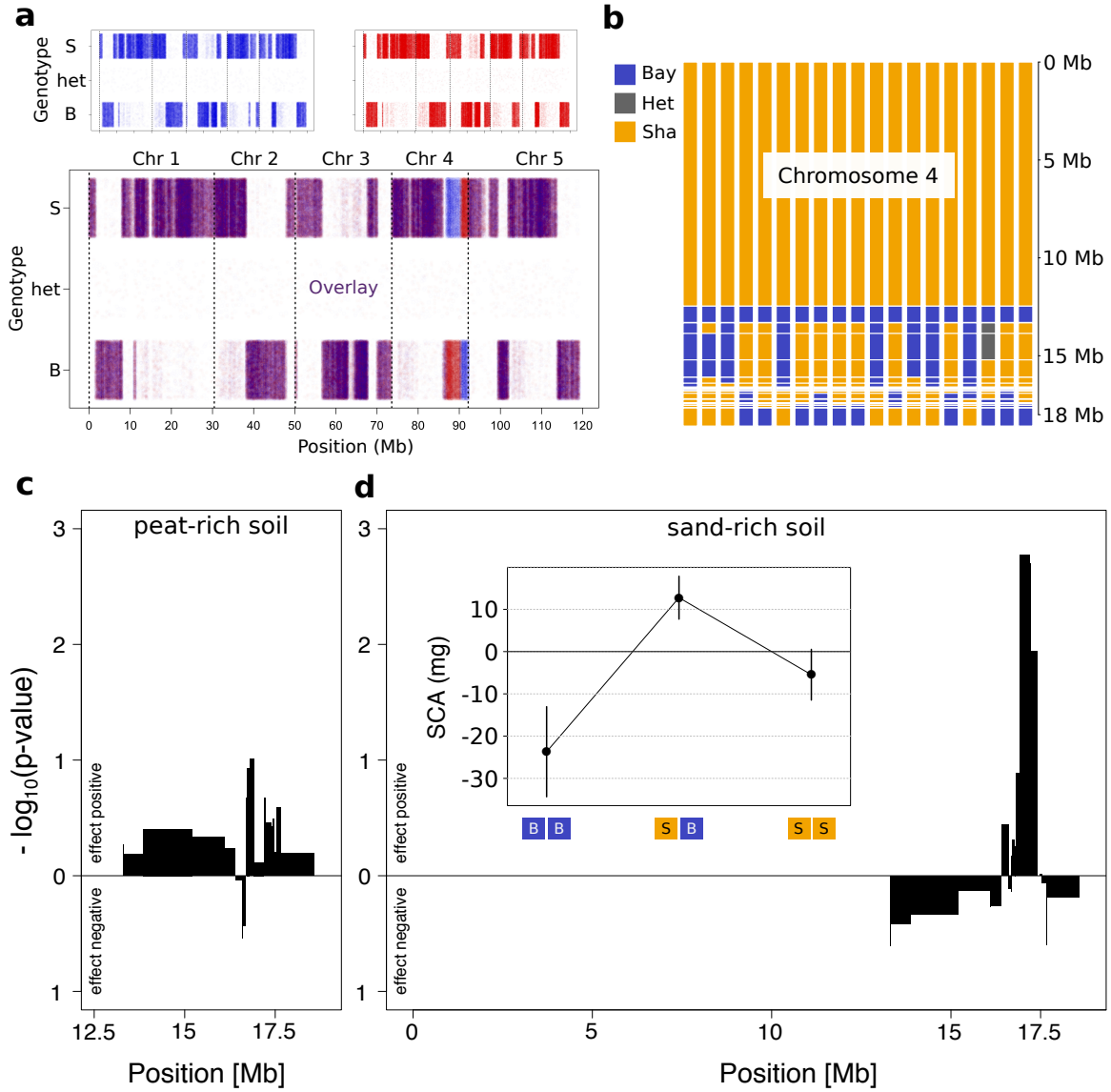


Figure 3 | Resolving soil × allelic diversity interactions to a single Mendelian factor. a, Re-sequencing of near-isogenic lines (NILs) differing only on lower arm of chromosome four for fine-mapping. Shown are genotype calls at all polymorphic sites across the genome (B = homozygous for the Bay allele, het = heterozygous, S = Sha allele) in either NIL r10 (blue, top left) or NIL r96 (red, top right), as well as an overlay of the two line's genotype calls (bottom). **b,** Reconstructed genotypes across chromosome four of the 19 NILs used for fine-mapping. Each bar represents a single NIL. **c, d,** Map of allelic diversity effects across chromosome four, on either peat-rich soil (c), or sand-rich soil (d). The widths of the bars indicate the size of the regions in which no

recombination events were inferred across the whole population. Inset in (d) shows the mean ± s.e.m. of SCAs across allelic compositions at the diversity effect locus.

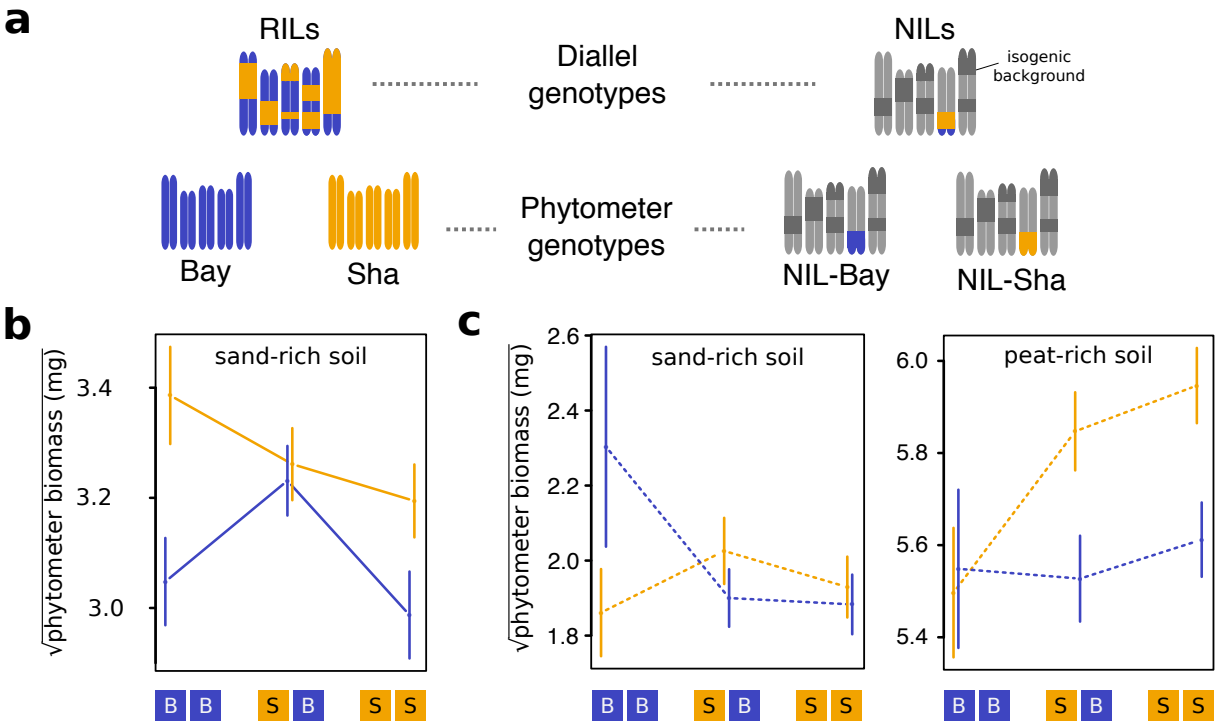


Figure 4 | Allelic diversity effects persist across a generation through their soil legacy. a, Scheme of the genotype-combinations used for either the recombinant inbred lines (RILs) vs. near-isogenic lines (NILs) diallels (top), and the phytometer genotypes used in the soil-feedback phase (bottom). **b**, Mean phytometer (Bay or Sha, +/- s.e.m.) performances on legacy soil derived from RIL mixtures with different allelic compositions at marker MSAT4.9. **c**, Mean phytometer (NIL-Bay or NIL-Sha, +/- s.e.m.) performances on legacy soil derived from NIL mixtures with different allelic compositions at locus Chr4@16.92 on either sand-rich (left) or peat-rich (right) soil. Values were square-root transformed for analyses.

Extended Data

Table S1 | Legacy effects of soils conditioned in RIL and NIL diallel experiments. Effects were quantified using two phytometers (factor 'phy'; Sha and Bay accessions in RIL diallel, and near-

isogenic lines bearing Sha and Bay allele at putative effect locus in NIL diallel). The NIL diallel additionally was replicated on substrates differing in sand content (factor 'soil'). The term 'GCA' (general combining abilities) indicates average genotype-specific soil conditioning effects on phytometer yields. 'SCA' (specific combining abilities) captures deviations in yield from additive predictions made using GCAs. Within SCA, the following contrasts were tested: GD: Genotype diversity, i.e. whether genotype monocultures differed in feedback effects from two-genotype mixtures; A: allele-specific differences at marker MSAT4.9 (RIL diallel) and Chr4@16.92 (NIL diallel) within genotype monocultures; AD: allele-diversity effects within genotype mixtures. df and ddf indicate nominator and denominator degrees of freedom of corresponding F-tests. *** P<0.001; ** P<0.01; * P<0.05; (*) P<0.1; n.s. not significant.

Terms and contrasts	RIL diallel			NIL diallel		
	df	Denominator: ddf	Signif.	df	Denominator: ddf	Signif.
GCA	18	comp: 167	(*)	18	comp: 168	n.s.
SCA						
GD	1	comp: 167	(*)	1	comp: 168	n.s.
A	1	comp: 167	n.s.	1	comp: 168	n.s.
AD	1	comp: 167	n.s.	1	comp: 168	n.s.
Phy × GCA	18	phy × comp: 166	n.s.	18	phy × comp: 168	*
Phy × SCA						
Phy × GD	1	phy × comp: 166	n.s.	1	phy × comp: 168	n.s.
Phy ×A	1	phy × comp: 166	n.s.	1	phy × comp: 168	n.s.
Phy ×AD	1	phy × comp: 166	**	1	phy × comp: 168	*
Soil × GCA				18	soil × comp: 150	n.s.
Soil × SCA						
Soil × GD				1	soil × comp: 150	n.s.
Soil ×A				1	soil × comp: 150	n.s.
Soil ×AD				1	soil × comp: 150	n.s.
Phy × Soil × GCA		does not apply		18	phy × soil × comp: 146	n.s.
Phy × Soil × SCA						
Phy × Soil × GD				1	phy × soil × comp: 146	***
Phy × Soil ×A				1	phy × soil × comp: 146	n.s.
Phy × Soil x AD				1	phy × soil × comp: 146	n.s.

Table S2: PCR markers used in this study.

Primer	Assay	Sequence	Predicted location	Pred. fragment sizes (Sha/Bay)
SW-182	ShaBa5	ACGTATTTCGATGTATGGTCCTTG	Chr4: 16044156	550/664
SW-183		TCACGTGAATCGTATTCGTTGAAG		
SW-184	ShaBa6	CTTCTCCGCTTCAACCTCTGC	Chr4: 17709750	600/632
SW-185		AATCCAGGATTCAGAGTTGCTTTC		
SW-188	ShaBa8	TTGATTAGGGCTACGAGGATAAGG	Chr4: 16707214	408/609
SW-189		GAGTCTATTAATTATGCTTGGTGC		

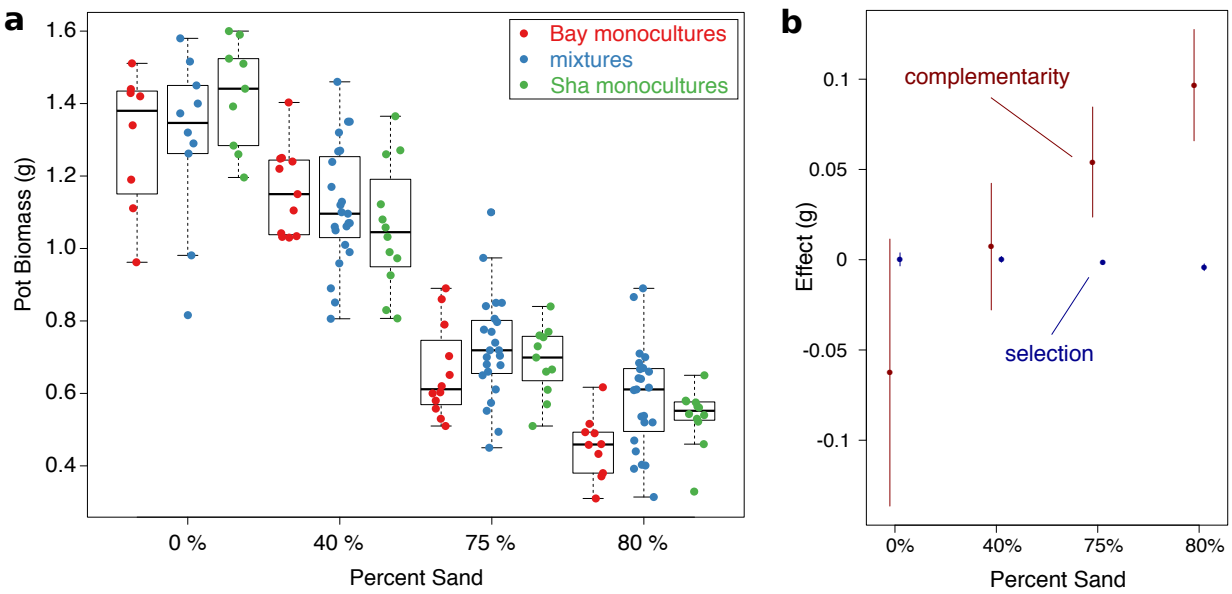


Figure S1 | Productivity and complementarity in Bay-Sha mixtures across a peat-sand gradient. a, Pot-level biomass measurements of Sha and Bay monocultures or pair-wise mixtures along a sand/peat substrate gradient. **b,** Complementarity and selection effects calculated according to the additive partitioning[8] method along the substrate gradient. Error bars denote s.e.m.

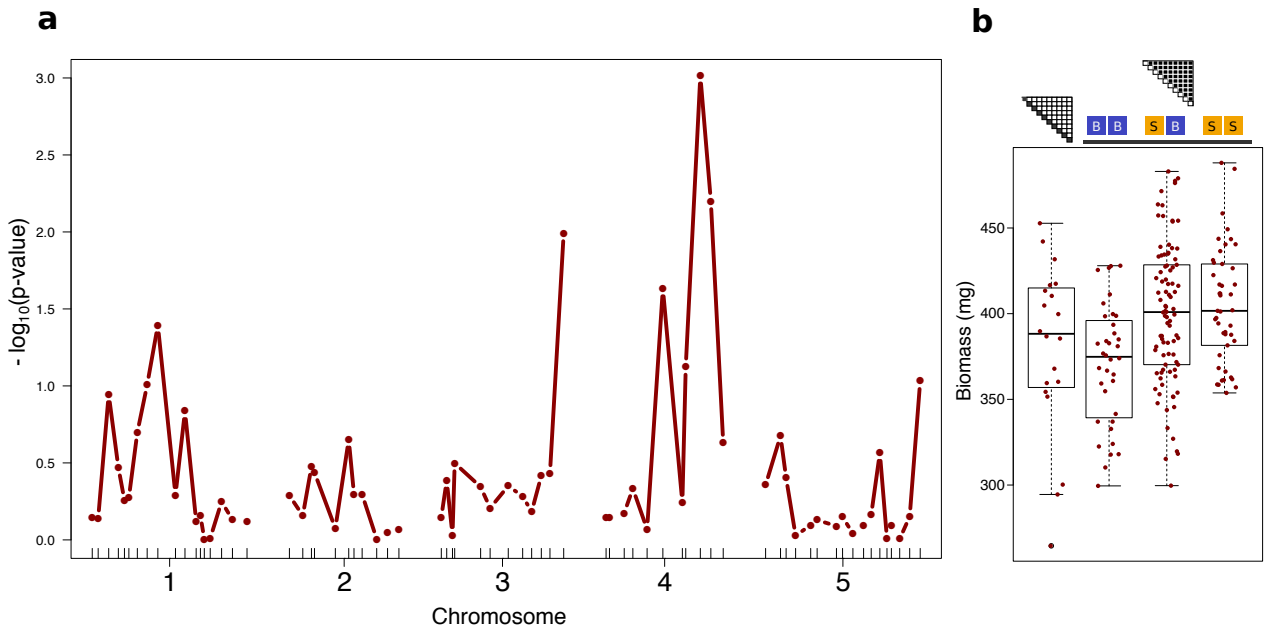


Figure S2 | A major effect locus driving complementarity between genotypes. a, QTL mapping of SCA variation across allelic diversity levels using a marker regression technique by contrasting SCAs of mono-allelic RIL mixtures (BB and SS) with bi-allelic mixtures (BS). **b**, Pot-level productivity of each genotype composition (average of four blocks) in dependence of allelic composition at marker MSAT4.9.

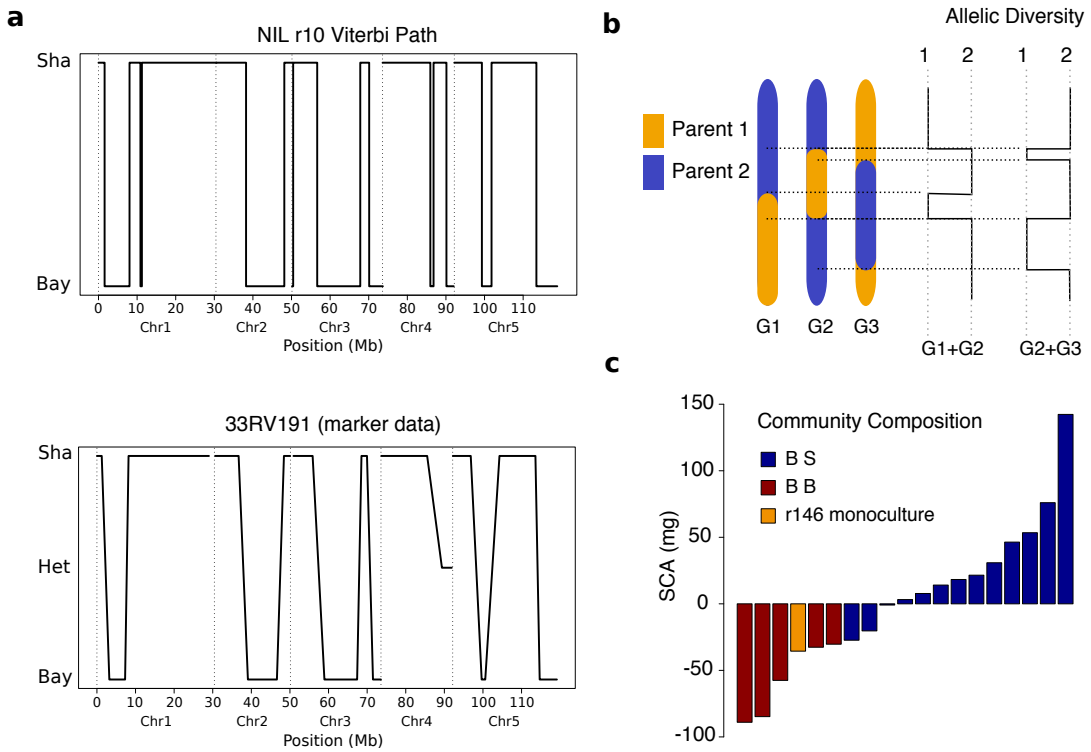


Figure S3 | Fine-mapping and persistence of allelic diversity effects in near-isogenic line. a, The comparison of the reconstruced genotype of NIL r10 (HMM Viterbi-path across all chromosomes, homozygous on lower arm of chromosome 4) in comparison to publicly available molecular marker-based genotyping data of the ancestral line from which it was derived (heterozygous on lower arm of chromosome 4) – showing a high degree of congruence between the re-constructed genotype based on whole-genome resequencing and the marker data. **b,** Schematic outline of a possible cause of the high mapping resolution achieved through the competition diallel design. A major advantage of the design is the joint dependency of community-level allelic diversity on recombinations *within* and *between* recombinant inbred lines, such that mapping resolution increases very quickly. **c,** Extreme example of SCA variation across genotype mixtures all containing one specific genotype (NIL r146, homozygous for the Bay-allele at locus Chr4@16.92), either in combinations with NILs carrying the Sha-allele (dark blue bars) or the Bay-allele (dark red bars). Shown are the data from sand-rich soil only.

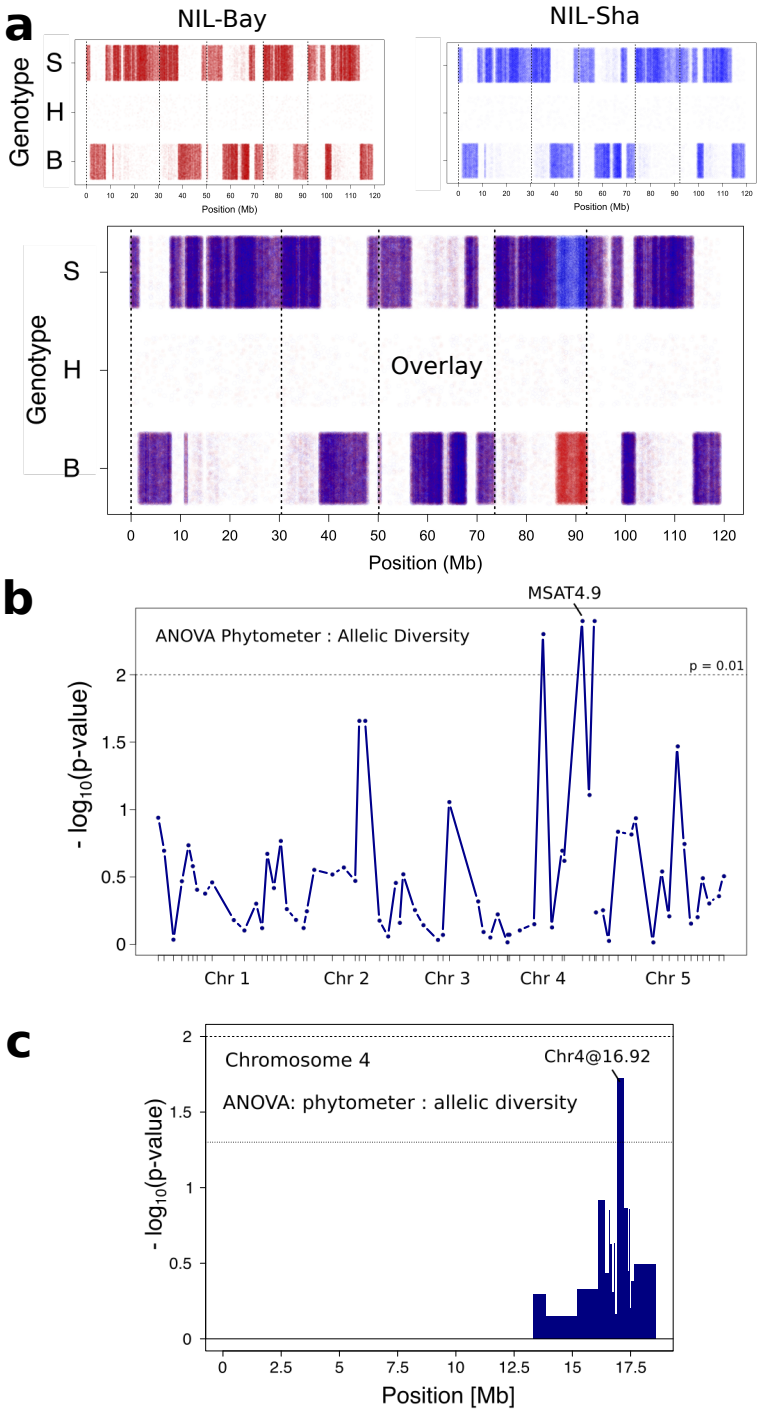


Figure S4 | Soil-feedback experiments. a, Genotype calls at all polymorphic sites across the genome (B = homozygous for the Bay allele, H = heterozygous, S = homozygous for the Sha allele) obtained by genome re-sequencing phytometer genotypes NIL-Bay (33RV191-Bay, top left, red) and NIL-Sha (33RV191-Sha, top right, blue). The overlay of the genotype calls of both lines (bottom) confirms that these lines are isogenic but for variation on lower arm of chromosome four.

The two phytometers were employed on soil derived from the NIL diallel. **b, c,** QTL mapping by marker regression of phytometer-specific responses to soil legacy of previous generation allelic diversity (i.e. allele-diversity × phytometer [Phy×AD] interaction in Supplemental Table S1, but in a model without adjusting for diallel pot biomass). The mapping of diversity effects through such influences on soil legacy could have been applied to the identification and fine-mapping of the same major effect locus, without ever measuring biomass productivity (albeit with slightly relaxed statistical criteria). Shown are negative $\log_{10}$-transformed P-values for each marker position in the RIL (b) or genotype block in the NIL (c) diallels.

Supplementary Discussion

As outlined in Figures 1 and 4, and as described in the Methods, we performed two soil-feedback experiments to test whether allelic diversity effects extend across generations after the plants were destructively harvested. Possible effects solely explainable by variation in plant productivity during the soil training phase (e.g. nutrient draw-down or environmental correlations) were accounted for in the model (terms *diallel biomass* and *diallel biomass×soil* as described in the Methods section). Significantly different soil conditioning through allelic diversity, as assessed by phytometer performance in a next generation, can thus be seen as an "extended phenotype" (i.e. a legacy of allelic mixture that extends throughout generations), and interactions thereof with phytometer genotype as asymmetric responses (potential trade-offs) of phytometers to soil-borne factors affected by the conditioning (term "Phy×AD" in Supplemental Table S1).

We noticed, however, that the exact mechanisms underlying both soil training or phytometer responses await further experiments, since the response patterns (Fig. 4) do not allow for a simple mechanistic model. One problem that impedes the inference of underlying mechanisms (such as the anticipation of specific soil factors, and their interaction with genetic variants) is the number of variables that differed between the two experiments because of constraints on experimental design:

in the RIL diallel, we used the two parental accession Bay and Sha as phytometers and a split-plot design to test for differential responses of these phytometer to soil conditioning. After the NIL diallel, we used two near-isogenic phytometer (NIL-Bay and NIL-Sha) as phytometers in a similar test for differential responses to soil conditioning. Naively, we could expect an approximate congruence of how the different phytometers carrying the same alleles at the diversity locus responded in the two experiments, i.e. the parental genotype Bay in the RIL diallel (Figure 4 b, yellow lines) should exhibit approximately the same response pattern to allelic diversity legacy as the NIL-Bay genotype in the NIL diallel (Figure 4 c, yellow lines). However, it seems for example that the Bay genotype grown on RIL diallel soil responded somewhat positively to conditioning by allelic diversity (a pattern opposite to what would be expected from simple biomass-corresponding resource drawdown during the diallel experiment - and indicating that diversity led to the increased "removal" of a negatively acting soil factor, or the addition of a positively acting soil factor), whereas the NIL-Bay genotype in the NIL diallel responded somewhat negatively to conditioning by allelic diversity. Variables differing between the experiments were 1) the environment (e.g. the different dates at which the experiments were conducted, different batches of soil, greenhouse conditions, etc), 2) the genetic variation in the two diallel population (RILs vs NILs), whereby the genetic variance due to epistasis is expected to differ strongly between these two population, and 3) phytometer genotypes (parental lines vs NILs). We chose to vary the phytometer genotypes because i) we did not have NILs available for the RIL diallel, yet ii) aimed to demonstrate that asymmetric soil legacy responses could arise in a near-isogenic background in the NIL diallel.

All these differences between the two soil-feedback experiments, alone or in combination, may explain why our naive expectation (outlined above) was not met. However, the experiments still convincingly show that i) allelic diversity effect extend across generations through soil conditioning, and ii) phytometer genotype determines the response to such conditioning in either experiment (phytometer×allelic diversity interaction, see Table S1). Furthermore, both of these

arguments apply to the NIL experiments, where the population-level genetic variation (both in the training and feedback phase) was restricted to a single region in an otherwise isogenic background. This suggests that positive productivity effects and soil conditioning are related by mechanism.