ECOLOGY LETTERS

Ecology Letters, (2014) **17**: 717–726 doi: 10.1111/ele.12276

LETTER

Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild Arabidopsis relative

Maggie R. Wagner,[1]* Derek S. Lundberg,[2] Devin Coleman-Derr,[3] Susannah G. Tringe,[3] Jeffery L. Dangl[2,4] and Thomas Mitchell-Olds[1,5]

Abstract

Plant phenology is known to depend on many different environmental variables, but soil microbial communities have rarely been acknowledged as possible drivers of flowering time. Here, we tested separately the effects of four naturally occurring soil microbiomes and their constituent soil chemistries on flowering phenology and reproductive fitness of *Boechera stricta*, a wild relative of Arabidopsis. Flowering time was sensitive to both microbes and the abiotic properties of different soils; varying soil microbiota also altered patterns of selection on flowering time. Thus, soil microbes potentially contribute to phenotypic plasticity of flowering time and to differential selection observed between habitats. We also describe a method to dissect the microbiome into single axes of variation that can help identify candidate organisms whose abundance in soil correlates with flowering time. This approach is broadly applicable to search for microbial community members that alter biological characteristics of interest.

Keywords

Flowering time, life history, microbiome, phenology, plant–microbe interactions, plasticity, selection, selective agents, soil ecology.

Ecology Letters (2014) **17**: 717–726

INTRODUCTION

Dissecting complex environments into biotic and abiotic components for individual study may lead to surprising and novel discoveries about how organisms respond phenotypically and evolutionarily to their habitats. This is because the net effect of a given environment is the accumulation of effects due to all relevant components of the ecosystem. Selection on floral characteristics, for instance, is mediated by many agents including pollinators, herbivores, temperature and water availability (Strauss & Whittall 2006). Thus, the complexity of natural habitats can preclude identification of the precise stimulus for phenotypic change or natural selection in the field (Anderson *et al*. 2014). By isolating simpler components of complex habitats, we can test whether each affects the phenotype expressed by a given genotype, the adaptive value of that phenotype, or both. These two effects of environment – phenotypic plasticity (Bradshaw 1965) and differential selection (Wade & Kalisz 1990) – are crucial to understanding trait evolution and fitness both in a historical context and in the context of a changing planet. Despite their importance, most ecological drivers of trait expression and natural selection are unknown (MacColl 2011). Because environmental effects are best understood as responses of one trait to a specific stimulus (Bradshaw 1965), disentangling the precise ecological interactions that cause plasticity and differential selection in nature is an important goal (MacColl 2011).

For plants, soil is a key component of the complex natural habitat. Soils contain intricate patterns of chemical, physical and microbial variation that are linked on continental (Fierer & Jackson 2006) and centimetre scales (Ettema & Wardle 2002; Berg & Smalla 2009). Feedbacks between above-ground plant communities, below-ground microbial communities and nutrient availability are common (Ettema & Wardle 2002; van der Heijden *et al*. 2008; Berg & Smalla 2009). At the level of populations and individual plants, soil microbes can affect plant growth (Rodríguez & Fraga 1999; van der Heijden *et al*. 2008), resistance to infection (Berendsen *et al*. 2012) and above-ground herbivory (Hol *et al*. 2010). In addition, microbes can mediate adaptation to novel environments (Lau & Lennon 2012). Subsets of the soil microbiome interact with plants by colonizing aerial plant tissues (Vorholt 2012) or the rhizosphere or root (Bulgarelli *et al*. 2012, 2013; Lundberg *et al*. 2012). Thus, soil chemistry, plant biology and microbial ecology are intricately linked (Ettema & Wardle 2002; Berg & Smalla 2009) and soils comprised many potential biotic and abiotic selective agents.

In this study, we investigated the role of soil as a driver of plasticity and as an agent of selection on flowering time, an important ecological trait for plants and their communities

[1]*Duke University Program in Genetics and Genomics, Department of Biology, Duke University, Durham, NC, 27708, USA*
[2]*Department of Biology, Howard Hughes Medical Institute, Curriculum in Genetics and Molecular Biology, Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC, 27599, USA*
[3]*Joint Genome Institute, Walnut Creek, CA, 94598, USA*
[4]*Department of Microbiology and Immunology, Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC, 27599, USA*
[5]*Department of Biology, Institute for Genome Sciences & Policy, Duke University, Durham, NC, 27708, USA*
**Correspondence: E-mail: maggie.r.wagner@gmail.com*

(Forrest & Miller-Rushing 2010). Flowering phenology has a strong genetic component (Brachi *et al.* 2010) but also responds to stimuli including temperature (Aikawa *et al.* 2010), water availability (Borchert *et al.* 2004; Crimmins *et al.* 2013), pathogen infection (Korves & Bergelson 2003) and herbivory (Brys *et al.* 2011). While soil chemistry is known to affect flowering time (Pigliucci *et al.* 1995; Stanton *et al.* 2000; Brun *et al.* 2003; Ryser & Sauder 2006), soil microbial communities have rarely been acknowledged as possible drivers of reproductive timing in plants. Furthermore, previous studies that explored the relationship between soil microbiome, flowering and selection used domesticated plants, artificial microbial communities, and/or biota from heavily disturbed soils (Batten *et al.* 2007; Lau & Lennon 2011, 2012). Although these experiments provide evidence that soil microbes change plant reproductive timing and selection pressures, they allow us to draw only limited conclusions about the evolutionary importance of this process.

Here, we asked whether (1) flowering phenology is plastic in response to different soil microbiomes, and (2) soil microbial communities alter the intensity of selection on flowering phenology. We further asked whether (3) relative abundance of specific members of the microbiome predict the observed effects of microbial treatments on flowering time. To test these hypotheses, we grew gnotobiotic (i.e. germinated in sterile conditions) seedlings of the non-mycorrhizal wild mustard *Boechera stricta* (Graham) Al-Shehbaz in sterilised potting soil inoculated with microbial communities extracted from soils of four undisturbed natural habitats. To enable comparison of biotic and abiotic soil variables, we also grew seedlings in field soils collected from the same habitats, which were sterilised to eliminate their natural microbiomes but retained their chemical and physical differences. For each individual plant, we recorded the day of first flowering, height, number of leaves and number of fruits. We quantified selection on phenology as the linear relationship between flowering time and fruit production.

Rather than manipulating microbial communities, we used presumably intact communities extracted from soils collected from undisturbed field sites near wild *B. stricta* populations. Our experiment included 48 natural, inbred accessions that represent the breadth of genetic diversity harboured by *B. stricta* in the study region (central Idaho, USA). Furthermore, we focused on a phenotype (flowering time) that is under selection in this species in a nearby field site (Anderson *et al.* 2011). To our knowledge, no other study has tested whether naturally occurring soil biotas from multiple undisturbed habitats affect flowering phenology and selection on flowering time, nor has any study of the relationship between soil microbes and phenology explicitly accounted for genetic variation among undomesticated plant populations.

MATERIAL AND METHODS

In summary, we sterilised potting soil to kill the resident microbiome, and then inoculated subsets of the sterilised potting soil with microbial communities extracted from soils from four different field sites. To create four additional treatments that captured abiotic soil variation, we sterilised the same four field-collected soils.

Sterilised potting soil was saturated with inoculum and sterilised field soils were saturated with buffer (see below) for 10 days. Then, 48 accessions of *B. stricta* were transplanted as gnotobiotic seedlings into all treatments (48 genotypes × 8 treatments × 4 replicates = 1536 individuals). All pots were randomised into blocks of 200 and maintained under controlled greenhouse conditions throughout the experiment, except for 7 weeks of 4 °C vernalization in a growth chamber. We measured plant height and leaf number on the date of first flowering. Here, we focus on phenology and fecundity of the 51% of plants that flowered successfully. Among those plants that flowered, the experimental design exhibited only modest imbalance, with substantial sample sizes remaining in every subspecies × treatment cell (mean $N = 54.6$, median $N = 56.5$). Factors influencing probability of flowering and other fitness components are beyond the scope of this study; we found no evidence that any experimental treatments affected flowering probability of surviving plants ($\chi^2 = 8.86$, $\text{d.f.}_{\text{num}} = 7$, $P = 0.26$). Reproductive fitness was estimated as the number of fruits on each individual at 33 weeks of age; in *B. stricta*, fruit set is strongly and positively correlated with seed production in the greenhouse ($R^2 = 0.72$, $P < 0.0001$, $N = 103$, A. Manzaneda 2008, unpubl. data).

Soil and seed collections

Soils were collected from four natural *B. stricta* habitats (described in Appendix S1) in central Idaho, USA, separated by ~26–92 km and differing in elevation, temperature, water availability, density and diversity of vegetation and many soil properties (M.R. Wagner, unpubl. data). Collection locations were named 'Jackass Meadow' (abbreviated 'JAM'), 'Mahogany Valley' ('MAH'), 'Parker Meadow' ('PAR'), and 'Silver Creek' ('SIL'). These remote sites have little history of disturbance by humans, are home to endogenous *B. stricta* populations and function well as common gardens for *B. stricta* field experiments (Rushworth *et al.* 2011). Therefore, they are legitimate potential habitats that *B. stricta* likely encountered during its evolutionary history in this region. Each soil collection (August 2012) comprised five subsamples from the four corners and approximate centre of a ~150 m^2 area, at a depth of ~10–30 cm. Subsamples were combined and mixed thoroughly, sieved through ~1.25 cm wire mesh to remove rocks and coarse detritus, shipped to Duke University and stored in plastic bags at 4 °C for ~3 months until further use. We also collected seven ~1 mL soil vouchers from each site for microbial community analysis: three in August 2011, and at all sites except PAR, four in August 2012. Vouchers were frozen at −20 °C until DNA extraction in late 2012.

B. stricta seeds were collected from 48 natural populations, including four from the soil collection sites. Their sites of origin span over 1000 m in elevation (Table S1) and are separated by between ~1 km and ~350 km, with the exception of the 'SAD12' genotype from Colorado. Because *B. stricta* is naturally inbred and exhibits high F_{IS} (i.e. individuals are largely homozygous) and F_{ST} (i.e. low genetic variation within populations and substantial divergence among populations; Song *et al.* 2006), each population was represented by a distinct genotype. This diverse collection of genotypes included

24 from each of the ecologically divergent 'east' and 'west' subspecies (Lee & Mitchell-Olds 2013). We used seeds from a single mother descended from the original field-collected accession, self-pollinated in the greenhouse for at least one generation, to minimise maternal effects; i.e. individuals within a genotype were self-full sibs (as in Anderson *et al.* 2011).

Microbial treatments in sterilised potting soil

To create four soils that were identical except for their microbial communities, we extracted microbes from field soils into sterile buffer and soaked sterilised potting soil in the resulting suspensions. We prepared inocula from 75 g subsamples of each field-collected soil stirred into 1 L of 2.5 mM MES monohydrate (Sigma Aldrich, St. Louis, MO, USA) in sterile diH_2O (pH adjusted to 5.7 with KOH). After settling for 30 min the suspensions were vacuum filtered (11-μm pore size) to remove particulates. Filtrates were centrifuged 30 min at 3000 ***g*** at room temperature to pellet microorganisms. To remove dissolved nutrients, we discarded the supernatants and resuspended the microbe-enriched pellets in 1 L sterile 2.5 mM MES. This process mostly eliminated variation in chemical properties that differentiate the field soils (Fig. S1, Table S2). Each rack of 200 pots was bottom saturated with 400 mL of one of the microbial suspensions, 6 g 20-10-20 fertiliser and sterile diH_2O for a total treatment volume of 4 L. The fertiliser was added to encourage seedling survival and to counteract possible soil impoverishment due to autoclaving (Berns *et al.* 2008). An additional 1 mL of undiluted microbial suspension was pipetted into each pot. Treatments derived by this process are termed 'biotic' or 'microbial treatments' throughout. It is possible that the filtration and recolonization processes somewhat altered community structures; however, the differences between our experimental inocula – and their effects on the plants – originate from corresponding differences between real *Boechera* habitats.

Sterilised field soil treatments

We sterilised soils from four natural habitats to create growth substrates with different physical and chemical properties, but without their natural microbiomes. After subsampling to extract microbial communities (see above), we sterilised the four field-collected soils via autoclaving (Appendix S2). These soils were loosely packed into clean pots and bottom saturated with 400 mL sterile 2.5 mM MES, 6 g 20-10-20 fertiliser and sterile diH_2O to bring the treatment volume to 4 L. An additional 1 mL sterile 2.5 mM MES was pipetted into each pot. Treatments derived by this process are termed 'abiotic' or 'sterilised field soils'. Although it is likely that autoclaving these soils changed their fertility, they appear to have retained at least some of their natural chemical variation (Fig. S1).

Plant care and trait measurement

Surface-sterilised seeds of 48 genotypes (Table S1) were stratified on autoclaved filter paper at 4 °C in the dark for 2 weeks, then placed in a growth chamber to germinate for 1 week (conditions in Appendix S2). Four germinated seedlings per genotype were transplanted into each of the eight experimental soils described above, one seedling per pot. Eight pots per treatment were left unplanted as controls. All pots were immediately rearranged into randomised blocks and maintained in controlled greenhouse conditions (Appendix S2) for the duration of the experiment. Plants were top watered as needed with RO water, and received an additional 4 mL 20-10-20 fertiliser (dissolved in sterile diH_2O) via pipet when 1 month old. Two-month-old plants were transferred to a 4 °C vernalization treatment, where they remained for 7 weeks.

After vernalization, plants were returned to the greenhouse, checked three times weekly for flowers and allowed to set fruit. Flowering was defined as sufficient separation of the corolla such that four distinct petals could be identified. The number of days between end of vernalization and first flowering is termed interchangeably 'flowering time' and 'flowering phenology' throughout. On the day of first flower for each plant we measured the individual's height (defined as the length of green tissue up to the apical meristem) and number of leaves. The last census was done 8 weeks post vernalization; the 749 plants that had not flowered by this date were excluded from all future analyses. The experiment ended 2 months after the final flowering census, when almost all fruits had matured and dehisced. These cut-offs for flowering time and fruit production are realistic given the short growing season observed in the field. At this time we counted the number of fruits produced by each individual.

Soil bacterial analyses

Due to current methodological limitations, in this study we focus on the prokaryotic component of the soil microbiome (i.e. bacteria and archaea). We extracted DNA from field-collected soil vouchers using the MoBio™ PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) and amplified variable region 4 of the bacterial 16S rRNA gene using established primer pairs 515F and 806R and PNA PCR clamps to reduce plastid and mitochondrial contamination (Lundberg *et al.* 2013). Paired-end 2 × 250 bp sequencing of barcoded amplicons was performed on a MiSeq machine running v2 chemistry (Illumina Inc., San Diego, CA, USA) at the Joint Genome Institute (Walnut Creek, CA, USA). The primer sequences were trimmed from the paired-end sequences, which were then overlapped and merged using FLASH (Magoč & Salzberg 2011). Merged sequences were grouped into operational taxonomic units (OTUs) based on 97% sequence identity, and chimeric sequences were removed, using the UPARSE pipeline (Edgar 2013). Taxonomies were assigned as in Lundberg *et al.* (2012). Unclassifiable OTUs at the kingdom level, OTUs matching Viridiplantae, mitochondrial, or plastid sequences were excluded by using BLAST to compare them to a custom database of contaminant sequences (Lundberg *et al.* 2013). Unclassifiable OTUs at the kingdom level and rare or non-reproducible OTUs were also excluded as in Lundberg *et al.* (2012), resulting in 7844 OTUs. To control for unequal sequencing effort, we normalised data by rarefaction to 40 000 reads/sample using QIIME-1.7.0 (Fig. S2; Caporaso *et al.* 2010). Diversity analyses were performed after correcting data for 16S gene copy number variation using scripts provided in Kembel *et al.* (2012); OTUs without taxonomic information

were assigned the mean copy number (2.805725). Linear regressions were performed before this correction but the resulting parameter estimates were adjusted as necessary.

Statistical analysis

To test the hypothesis that soil properties affect flowering time, we used restricted maximum likelihood (REML) linear mixed models with treatment, subspecies and treatment × subspecies as fixed factors; block, genotype nested within subspecies and treatment × genotype as random factors; and elongation rate (mm/day), height at first flowering (mm) and leaves per mm stem as covariates (justification in Appendix S2). To test for treatment effects on overall plant size we used multivariate analysis of variance (MANOVA) with treatment, subspecies and treatment × subspecies as fixed factors. We analysed the parallel 'biotic' and 'abiotic' experiments separately, i.e. one model tested only for effects of soil microbiomes on flowering time, and another identical model tested only for effects of physical soil differences; we did not directly compare the effects of these two types of soil variation. These models were run using JMP® Pro version 10.0.0 (SAS Institute, Cary, NC, USA). Statistical significance of random effects was determined by REML likelihood ratio test and results were graphed using *ggplot2* (Wickham 2009) in R version 3.0.2 (R Core Team 2013).

To test the hypothesis that soil properties alter selection on flowering time, we used a REML linear mixed model with the same terms as above, plus flowering time (days after vernalization) and flowering time × treatment as additional fixed effects. The response variable was number of fruits. Thus, the flowering time term describes the change in fecundity attributed to a change in flowering time, i.e. selection. We analysed the parallel 'biotic' and 'abiotic' experiments separately as above. We performed these models both with and without including elongation rate, height at first flowering and leaves per mm stem as covariates; the former model describes the *selection gradient* on flowering time (i.e. direct selection on flowering time independent of selection on covariates), and the latter model describes the *selection differential* (i.e. total selection on flowering time, including indirect effects of selection on covariates). Introducing a quadratic term did not improve fit, so our model considers only linear effects (i.e. directional selection). Sample sizes were slightly smaller than for the flowering time models because 17 individuals were accidentally discarded after flowering. Main models were performed in JMP; selection differentials and selection gradients were calculated in R.

For some models, non-uniformly distributed residuals might have influenced judgments about significance. This heteroscedasticity resisted data transformation and resolved only when covariates were removed. In general, results with and without covariates were similar, suggesting that significance was robust to heteroscedasticity in the standard model. For the sake of caution, for all of our major results, we performed permutation tests (which do not assume uniform residuals) to verify the results of the standard analysis of variance (ANOVA).

Rarefied microbial communities were analysed in the R package 'vegan' (Oksanen *et al.* 2013). Principal coordinates (PCo) of Bray–Curtis pairwise dissimilarities were identified using the vegan function 'capscale'. Similarity of samples within vs. among sites was tested using the non-parametric permutation test ADONIS with 9999 permutations constrained by collection year. To ask which components of microbial communities affect flowering phenology and selection, we regressed mean flowering time in each biotic Treatment (using residuals from the linear models described above in Table 1a, excluding terms with the 'Treatment' factor) onto the mean PCo score from the corresponding site. To ask which OTUs underlie the observed phenotypic effect, we identified the 10 OTUs most highly correlated with the PCo axes and regressed the same flowering time residuals on the OTUs' mean abundances at each site. We used the Wilcoxon rank-sum test to compare relative abundances of common taxa between groups of samples associated with extreme phenotypes. *P*-values were adjusted using Benjamini–Hochberg false discovery rate. Our method of searching for microbial

Table 1 Statistics for REML mixed models of flowering time for (a) soil microbial community treatments and (b) sterilised field soil treatments

	(a) Soil microbial communities (N = 451, Adj. R^2 = 0.74)			(b) Sterilised field soils (N = 336, Adj. R^2 = 0.72)		
	d.f.	F or χ^2	P	d.f.	F or χ^2	P
Treatment	3,87	4.242	**0.0076***	3,96	6.586	**0.0004**
Subspecies	1,67	6.861	**0.0109**	1,64	0.085	0.7717
Treatment × subspecies	3,88	2.346	0.0782	3,91	0.206	0.8919
Genotype (ssp.)	2	30.08	**< 0.0001**	2	0.864	>0.05
Geno. (ssp.) × treatment	6	0.000	>0.05	6	0.160	>0.05
Block	1	0.083	>0.05	1	0.785	>0.05
Elongation rate	1,428	304.2	**< 0.001**	1,278	342.9	**< 0.0001**
Height at flowering	1,387	239.0	**< 0.001**	1,240	278.1	**< 0.0001**
Leaves per mm stem	1,428	15.96	**< 0.001**	1,298	21.38	**< 0.0001**

All effects are fixed except for Block, Genotype (ssp.) and Genotype (ssp.) × Treatment. For fixed effects the test statistic F is reported. For random effects, the test statistic χ^2 is reported, calculated as twice the difference between log likelihoods of the full model and the model with the random factor excluded. A similar model that did not control for growth-related covariates yielded similar results (Table S4). Parameter estimates are listed in Table S4. Bold type indicates significance of at least $P < 0.05$.

*Permutational ANOVA confirmed significance at $P = 0.005$.

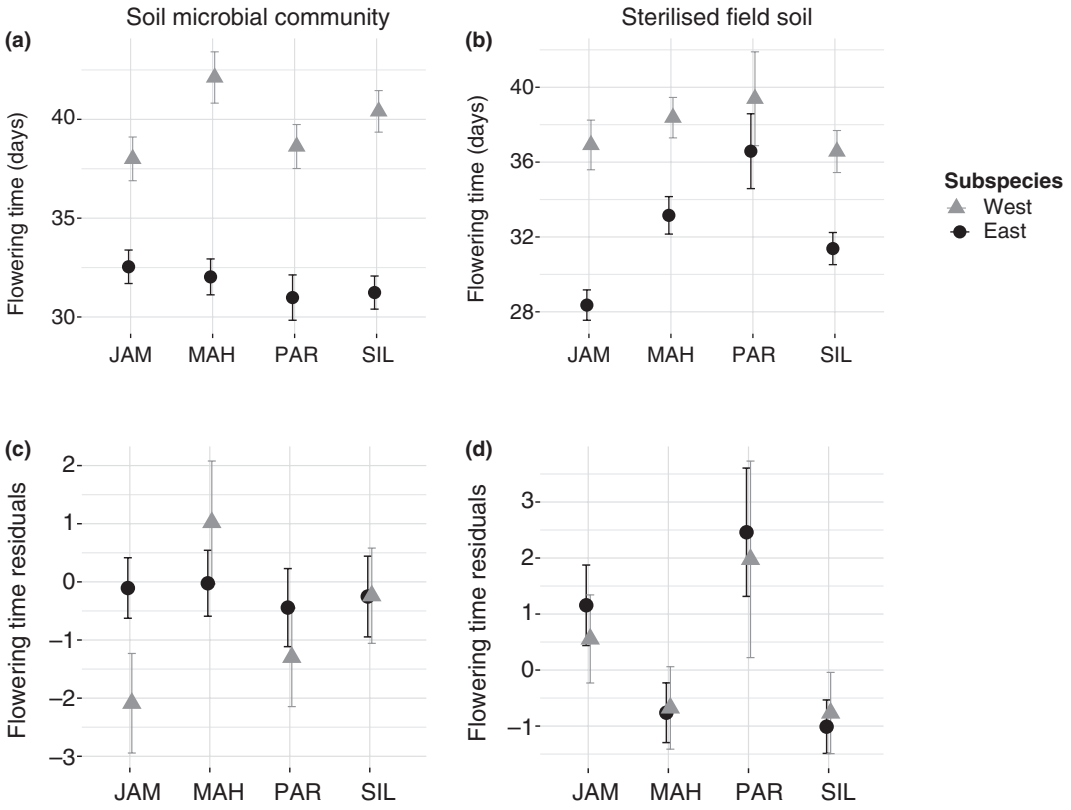

Figure 1 Variation in both soil microbiota and soil chemistry alters flowering phenology. Mean flowering time post vernalization (raw data, in days) is shown for each subspecies in (a) four microbial treatments and (b) four sterilised field soils. (c) and (d) show mean flowering time residuals, after controlling for genetic differences and growth covariates, for each subspecies in biotic and abiotic treatments respectively. Error bars depict one standard error from the mean. Parameter estimates and standard errors are reported in Table S3.

community members that underlie our phenotype of interest is described in more detail in Appendix S3.

RESULTS

Microbial communities and physical soil types each alter mean flowering time

Soil microbiota altered mean flowering time across all genotypes (Fig. 1a; Table 1a; $F_{3,87} = 4.242$, $P = 0.008$; permutational ANOVA $P = 0.005$). In particular, the MAH microbiome delayed flowering by 2.2 days on average (or 1.5 days when controlling for plant size and growth rate; Table S3). This delay translates to a 9% fitness decrease based on the selection differential measured at a similar field site (Anderson *et al.* 2011). Although western genotypes appear more sensitive to microbes than eastern genotypes (Fig. 1a, c), the subspecies difference in microbiome-driven plasticity of flowering time was not significant (Treatment × subspecies: $F_{3,88} = 2.346$, $P = 0.078$). Genotype explained 34.5% of the variance in flowering time, indicating substantial among-population genetic variation for flowering phenology across all microbiomes ($\chi^2 = 30.08$, $\text{d.f.}_{\text{num}} = 2$, $P < 0.0001$). In contrast, genotype × treatment explained no variation, suggesting a lack of genetic diversity for flowering time plasticity in response to soil microbiota. Excluding growth-related covariates from the model did not qualitatively change the results (Table S4a). Neither soil microbiome (MANOVA Wilks' $\lambda = 0.982$, $F_{9,1073} = 0.90$, $P = 0.53$) nor subspecies × soil microbiome (MANOVA Wilks' $\lambda = 0.976$, $F_{9,1073} = 1.21$, $P = 0.28$) affected overall plant size.

Differences between sterilised field soils also affected flowering phenology (Figs. 1b, d; Table 1b; $F_{3,96} = 6.586$, $P = 0.0004$). PAR soil had the most extreme effect, delaying flowering by 2.8 days after controlling for effects on growth. Again, the subspecies did not differ in their flowering time response to soil type (Fig. 1d; $F_{3,91} = 0.206$, $P = 0.8919$). Genotype explained only 5.7% of the variance in flowering time ($\chi^2 = 0.864$, d.f. = 2, $P > 0.05$) and genotype × treatment explained 4.72% ($\chi^2 = 0.160$, d.f. = 6, $P > 0.05$), indicating scant genetic variation for flowering time plasticity in response to abiotic soil variation. However, genetic effects were more pronounced when growth covariates were excluded from the model (Table S4b).

Microbial community, but not soil chemistry, alters selection on flowering time

Selection on flowering time depended on soil microbiome. This result held both for selection gradients (independent of selection on correlated traits; Fig. 2c, Table 2a; $F_{3,246} = 3.489$, $P = 0.016$; permutational ANOVA $P = 0.024$) and selection differentials (net selection on flowering time,

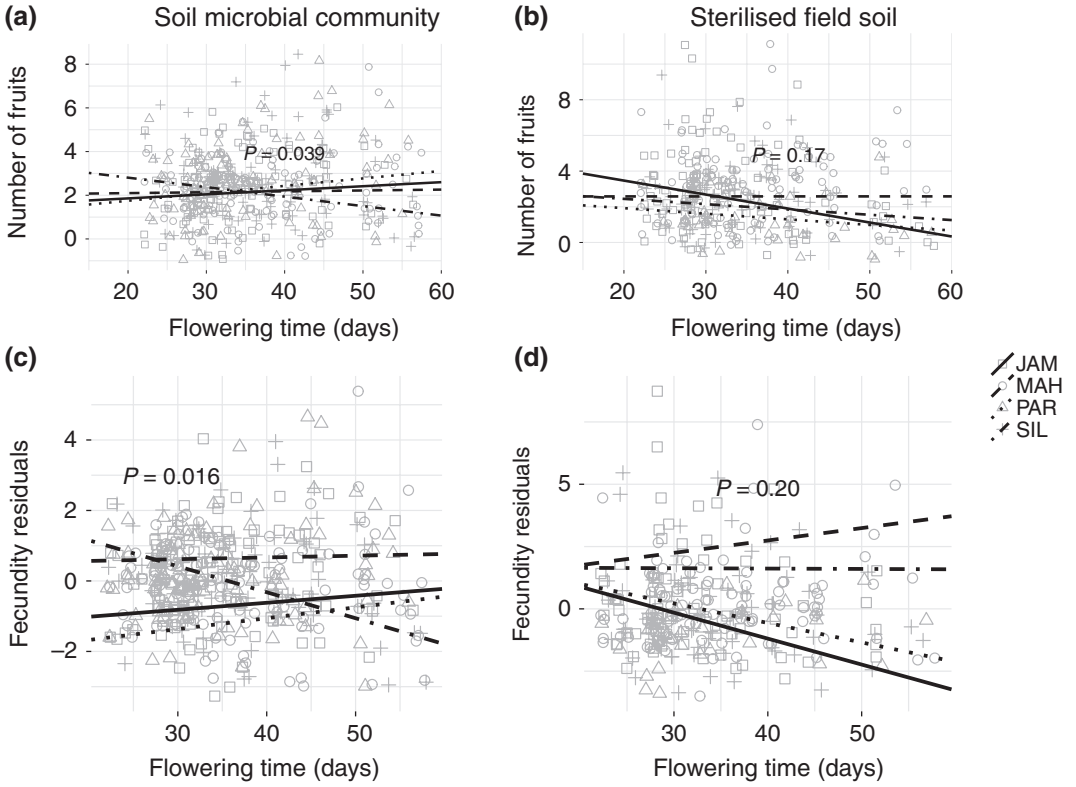

Figure 2 Different soil microbiota, but not sterilised field soils, alter selection on flowering time. Fecundity as a function of flowering time is shown for four different (a, c) soil microbial communities and (b, d) sterilised field soils. (a) and (b) depict selection *differentials* on flowering phenology, i.e. both direct selection on flowering time and indirect selection on correlated traits, after controlling for block effects and genetic differences in fitness. The statistics for the selection differential model are in Table S5. (c) and (d) depict selection *gradients* on flowering phenology, i.e. only direct selection on flowering time after controlling for selection on growth-related covariates in addition to block effects and genetic differences in fitness (statistics in Table 2).

including indirect effects of selection on covarying traits; Fig. 2a, Table S5a). The most extreme change was between the PAR and SIL soil biotas (Fig. 2a), with selection differentials of + 0.034 and − 0.043 fruits per day (or + 1.4% and − 1.8% fruits per day) respectively. The magnitude of this difference in selection is 1.2 times the selection differential measured in a nearby field site (Anderson *et al.* 2011). We detected no directional selection on flowering time when averaged across all treatments ($F_{1,403} = 0.009$, $P = 0.924$), suggesting that treatment differences in selection intensity cancelled each other out. On average, western genotypes produced 0.75 more fruits than eastern – a 27% increase ($F_{1,69} = 7.351$, $P = 0.008$; Table S3). In addition, genetic differences among populations within subspecies contributed 20.4% of the variance in fecundity ($\chi^2 = 13.79$, d.f. = 2, $P < 0.01$). However, the lack of subspecies × treatment and genotype × treatment interactions shows that genetic effects on performance were not microbiota dependent. Finally, fitness was consistent across microbial treatments ($F_{3,86} = 2.123$, $P = 0.103$), indicating no net effect of different microbiomes on fecundity (Tables 2a, S3).

In contrast, we found no evidence that different sterilised field soils affect flowering time selection differentials (Fig. 2b; $F_{3,301} = 0.809$, $P = 0.17$) or gradients (Fig. 2d; $F_{3,305} = 1.552$, $P = 0.20$). However, these treatments affected overall fecundity ($F_{3,110} = 5.849$, $P = 0.001$; Table S3), indicating strong differences in soil quality. Western genotypes produced 69% more fruits than eastern genotypes on average ($F_{1,64} = 16.13$, $P = 0.0002$), but this advantage was consistent across all soils (Table S3). We detected no net selection gradient on flowering time across all field soils ($F_{1,308} = 0.376$, $P = 0.540$); however, we did find a significant selection differential on flowering time (− 0.036 or − 1.4% fruits per day; $F_{1,313} = 6.473$, $P = 0.011$; Table S5).

Separation of microbiome components reveals finer details of selection and phenology control

Sampled soil microbiomes differed between sites within year ($R^2_{ADONIS} = 0.29$, $P = 0.003$) but not between years within site ($R^2_{ADONIS} = 0.03$, $P = 0.56$). These results are supported by PCo analysis, in which samples clustered mainly by site (Fig. 3a). The first three PCo cumulatively explained 69.8% of prokaryotic community variation (Fig. S3). Because the MAH and JAM soils, which had the slowest and fastest flowering times, respectively, in our experiment, were separated primarily on the PCo2 axis (Fig. 3a), PCo2 became our candidate microbiome component to test for effects on flowering time. Because eastern genotypes appeared insensitive to microbes (Fig. 1c), we used flowering time residuals of western genotypes as the response variable. We did not find a significant relationship between mean flowering time in each treatment

Table 2 Statistics for REML mixed models of reproductive fitness for (a) soil microbial community treatments and (b) sterilised field soil treatments.

	(a) Soil microbial communities (N = 439, Adj. R^2 = 0.26)			(b) Sterilised field soils (N = 331, Adj. R^2 = 0.47)		
	d.f.	F or χ^2	P	d.f.	F or χ^2	P
Treatment	3,86	2.123	0.1032	3,110	5.849	**0.0010**
Subspecies	1,69	7.351	**0.0085**	1,64	16.13	**0.0002**
Flowering time	1,403	0.009	0.9240	1,308	0.376	0.5399
Treatment × subspecies	3,97	2.053	0.1115	3,109	1.170	0.3247
Treatment × flowering time	3,246	3.489	**0.0164***	3,305	1.552	0.2011
Genotype (ssp.)	2	13.79	**< 0.01**	2	0.145	>0.05
Geno. (ssp.) × treatment	6	0.0	>0.05	6	4.081	>0.05
Block	1	0.0008	>0.05	1	0.130	>0.05
Elongation rate	1,376	0.0780	0.7801	1,300	0.1829	0.6692
Height at flowering	1,385	3.116	0.0783	1,298	0.1291	0.7196
Leaves per mm stem	1,381	15.03	**0.0001**	1,303	0.1157	0.7339

All effects are fixed except for Block, Genotype (ssp.) and Genotype (ssp.) × treatment. For fixed effects the test statistic F is reported. For random effects, the reported test statistic is χ^2, calculated as twice the difference between log likelihoods of the full model and the model with the random factor excluded. Note that in this model 'flowering time' reflects the selection gradient, i.e. direct selection on flowering time after controlling for selection on growth-related covariates. A similar model without growth covariates revealed that the selection differential, i.e. a measure of combined direct and indirect selection on flowering time, behaves similarly with respect to the flowering time × treatment interaction (Table S5). Parameter estimates are listed in Table S3. Bold type indicates significance of at least $P < 0.05$. *Permutational ANOVA confirmed significance at $P = 0.024$.

and the mean PCo2 score of soil samples from the corresponding site (Fig. 3b; $F_{1,2} = 2.362$; $P = 0.26$). Likewise, none of the individual OTUs with high PCo2 scores predicted flowering time (Table S6); for instance, abundance of OTU_96997 (Hyphomicrobiaceae) appeared to correlate with phenology but the relationship was non-significant after correction for multiple comparisons (Fig. 3c; $F_{1,2} = 25.74$; $P = 0.037$; $P_{FDR} = 0.38$). These negative results may be due to lack of power caused by our sampling scheme ($N = 4$; Appendix S3).

The phyla Proteobacteria and Crenarchaeota were more abundant, and Acidobacteria were less abundant, in slow-flowering MAH compared to fast-flowering JAM soil communities (Table 3b). Within phyla, several families – including Koribacteraceae, Solibacteraceae, Opitutaceae, Verrucomicrobiaceae, Solirubrobacteraceae and Mycobacteriaceae – differed in relative abundance between MAH and JAM soil communities (Fig. S4, Table S7). In addition, Verrucomicrobia and Gemmatimonadetes were enriched in the 5% of OTUs with the highest loadings on PCo2 compared to the full natural communities (Table 3c), indicating that these phyla contribute disproportionately to the microbiome variation summarised by PCo2. These 'candidate taxa' are promising organisms for further study of microbial influences on flowering time.

DISCUSSION

Our analysis of native plant genotypes and soil microbial communities from four undisturbed environments suggests that soil microbiomes contribute to the ecology and evolution of flowering phenology in *B. stricta*. First, we showed that soil microbiota influenced phenotypic plasticity of flowering time. Second, soil microbiota altered the strength and direction of selection on flowering time. Finally, we showed how this type of experiment could be combined with quantitative descriptions of soil community composition to search for microbial species that affect important phenotypes. Our results show that experimental dissection of complex environments can reveal the ecological interactions shaping phenotypic expression and natural selection.

Our finding that both soil microbes and soil chemistry cause plasticity of flowering time agrees with previous reports, although to our knowledge only one other study has tested the effects of both biotic and abiotic components of the same soil. Lau & Lennon (2012) found that *Brassica rapa* flowered faster in dry conditions, and that a soil microbial community with a history of drought stress accelerated flowering compared to the wet-adapted replicate of the same community. Other soil properties reported to delay flowering include heavy metal concentration (Brun *et al.* 2003; Ryser & Sauder 2006), nutrient depletion (Pigliucci *et al.* 1995; Stanton *et al.* 2000), high salinity (Van Zandt & Mopper 2002) and a history of invasive plant growth (Batten *et al.* 2007). Notably, some species' phenology may be more robust than others' in response to soil heterogeneity (Batten *et al.* 2007). In general, such phenotypic plasticity has important ecological and evolutionary consequences (Bradshaw 1965; Thompson 1991; Miner *et al.* 2005; Richards *et al.* 2006; Ghalambor *et al.* 2007). For *B. stricta* in particular, phenological plasticity affects the plants' ability to time reproduction for optimal seed set (Anderson *et al.* 2011). In fact, the exclusion of natural soil microbiomes from growth chamber replicates of that experiment might be partially responsible for the low genetic correlation of flowering time in the field and in the laboratory (Anderson *et al.* 2011). Interestingly, our data hint that West genotypes may be more sensitive to soil microbiome than East genotypes ($P = 0.078$; Fig. 1a, c; Table 1a), suggesting intraspecific genetic variation for microbe-induced flowering time plasticity. In contrast, the two subspecies show very similar sensitivities to abiotic soil properties (Fig. 1d), indicating that flowering time plasticity to these two stimuli may have different genetic bases.

Typically, selection analyses allow us to infer and compare the adaptive value of particular traits in particular environments, but do not tell us why differential selection exists.

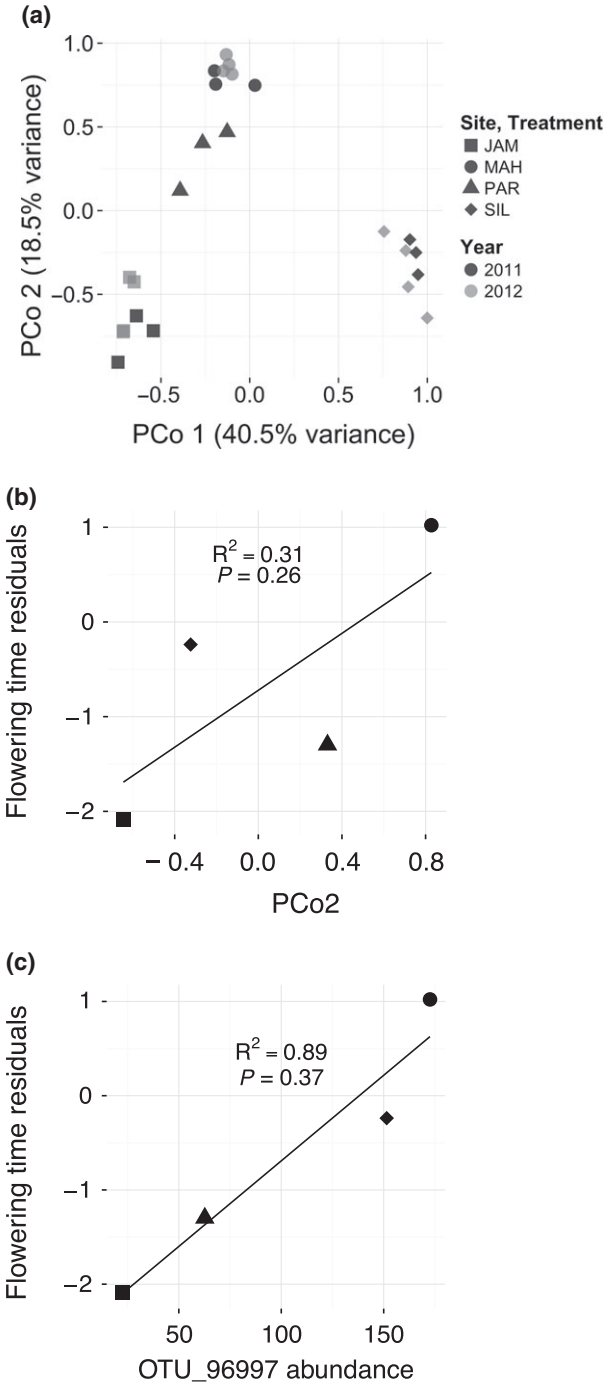

Figure 3 (a) Ordination of Bray–Curtis dissimilarities of rarefied microbial abundances shows clustering of soil samples by field site of origin (ADONIS $P = 0.003$), but not by collection year (ADONIS $P = 0.56$). (b) Flowering time response of western genotypes (residuals, after controlling for block effects, genetic differences and growth rate) to a gradient of soil microbiome PCo2, a candidate predictor of flowering time. (c) Flowering time response of western genotypes [residuals, as in (b)] to abundance of candidate microbe OTU_96997, chosen for its high loading onto PCo2 (Table S6; Appendix S3).

Here, we identified the soil microbiome as an agent of selection on flowering time in *B. stricta* (Figs. 2a, c). The evolutionary relevance of this finding is best illustrated by comparison with Anderson *et al.* (2011), who measured a decrease in fecundity of 0.06 fruits per day to flowering (> 4% of total mean fitness per day) in a typical *B. stricta* habitat. Our results suggest that all else remaining constant, a change in soil microbiome could increase or decrease that selection differential by up to 0.07 fruits per day, or > 120% (based on the magnitude of differential selection measured in the PAR and SIL soil communities; Fig. 2a). The effects of the soil microbiome on both flowering time and its adaptive value may prove especially important in the context of conservation and adaptation to global change, given that both microbial communities and plant phenology are sensitive to climate (Cleland *et al.* 2007; Castro *et al.* 2009).

Although we are not the first to report that soil biotas affect flowering time (Lau & Lennon 2012) or selection on flowering time (Lau & Lennon 2011), our experiment adds to previous work in several unique ways. First, we used deep 16S rDNA sequencing methods to greatly enhance our resolution of the microbiome (Lundberg *et al.* 2013). Second, we described how this type of microbiome data can be combined with experimental phenotype data to determine which microbial taxa influence traits of interest. Third, including a diverse set of genotypes in the experiment allowed us to test for intraspecific genetic variation for flowering time plasticity in response to biotic and abiotic soil attributes, and link this ecological finding to the field of evolutionary biology. Finally, we are the first to use microbial communities from several undisturbed field sites to confirm that naturally occurring variation in soil microbes affects phenology and its adaptive value. We identified several taxa that are enriched or depleted in soils associated with fast flowering compared to slow flowering (Tables 3b, S4); these groups are promising targets for future study of the microbe-flowering time relationship.

As evolutionary biologists, a fundamental goal is to measure selection and trait expression in the field because the genotype–phenotype–fitness relationship is frequently context dependent (Anderson *et al.* 2014). In particular, the true relationships between plants and microbial communities may depend on neighbouring plants (Berg & Smalla 2009) or on other soil properties (van der Heijden *et al.* 2008). For instance, Lau & Lennon (2012) found that the interaction between microbes and soil moisture synergistically affects fitness (but not phenology) in *Brassica rapa*. Factorial application of a wider sampling of microbiomes and environmental variables – or, eventually, direct manipulation of these variables in the field – would be especially informative for understanding the ecological mechanisms of plant–microbe interactions such as the flowering time effect we observed in *B. stricta*. Nonetheless, we demonstrate here that greenhouse experiments can reveal ecological interactions that may have been extremely difficult to detect directly in the field: all else remaining equal, natural differences in the soil microbiome can influence plant phenology and patterns of selection. This discovery required the isolation of soil microbiota from the larger, more complex natural habitat. Further environmental simplification – the reduction of the microbiome into PCos and then specific OTUs – potentially could reveal even more details of the relationship between genotype, phenotype and the microbial environment (Appendix S3). Although in this experiment we lacked power to fully utilise this method, it holds promise for future studies on the phenotypic

Table 3 (a) Mean flowering time of western genotypes (residuals after controlling for genetic differences and growth rates), mean PCo2 score and mean abundance of OTU 96997 in each microbial community. Flowering time data come from the greenhouse experiment, community composition data come from soil samples taken from the same field sites as the inocula for the greenhouse experiment. This set of data can be informative for finding subsets of the microbial community associated with change in flowering time (Fig. 3; Appendix S3). (b) Copy number-adjusted relative abundances of dominant phyla in slow-flowering MAH and late-flowering JAM soils. (c) Copy number-adjusted relative abundances in the full dataset ('All OTUs') and the subset of 5% of OTUs most strongly correlated with PCo2, a putative predictor of flowering time

(a) Site	Flowering time (residual)	PCo2	OTU 96997 (individuals, ± 1 SE)
JAM	−2.088 ± 0.856	−0.646 ± 0.068	22.27 ± 4.04
MAH	1.022 ± 1.057	0.827 ± 0.024	172.60 ± 16.67
PAR	−1.298 ± 0.848	0.331 ± 0.107	62.67 ± 12.90
SIL	−0.238 ± 0.819	−0.324 ± 0.068	151.47 ± 49.94

Phylum	(b) JAM	MAH	P_{FDR}	(c) All	PCo2	P_{FDR}
Proteobacteria	17.3%	26.7%	**0.002**	24.8%	23.5%	0.49
Acidobacteria	21.3%	15.3%	**0.002**	17.6%	19.0%	0.40
Verrucomicrobia	19.3%	15.3%	0.054	17.0%	22.5%	**0.004**
Actinobacteria	16.0%	17.8%	0.21	16.0%	14.3%	**0.036**
Gemmatimonadetes	9.5%	9.4%	1	7.7%	9.6%	**0.036**
Chloroflexi	5.7%	7.3%	0.054	6.0%	5.5%	0.40
Planctomycetes	3.4%	2.8%	0.054	4.0%	0.43%	**< 0.001**
Bacteroidetes	2.9%	2.7%	1	3.7%	2.4%	**0.012**
Crenarchaeota	0.06%	1.5%	**0.002**	0.70%	0.95%	0.61

Significance of enrichment/depletion was determined by Wilcoxon Rank-Sum tests and adjusted using the Benjamini–Hochberg false discovery rate.

effects of the environmental microbiome. This approach is generally applicable to search for microbial community members that alter biological characteristics of interest.

ACKNOWLEDGEMENTS

M.R.W. was supported by a Graduate Research Fellowship and a Doctoral Dissertation Improvement grant from the National Science Foundation and NIH Training grant 5T32 GM007754-32. T.M.O. was supported by grant R01 GM086496 from the National Institutes of Health and EF-0723447 from the National Science Foundation. J.L.D. was supported by NSF Microbial Systems Biology grant IOS-0958245 and DOE Plant Feedstocks grant DOE-SC0010423. D.S.L. was supported by NIH Training grant T32 GM07092-34. J.L.D. is an Investigator of the Howard Hughes Medical Institute and support was provided by the Howard Hughes Medical Institute and the Gordon and Betty Moore Foundation (in part via grant GBMF3030 to J.L.D.). This work was also funded by the JGI Director's Discretionary Grand Challenge Program, and a JGI community Sequencing Project (S.G.T., J.L.D. and T.M.O.). Work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. Many thanks to L.T. Burghardt and three anonymous reviewers for helpful comments and suggestions on earlier versions of the manuscript, and to J. Mays and the Duke greenhouse staff for watering assistance.

AUTHORSHIP

M.R.W. collected soils, designed and executed the experiment, analysed data, wrote permutation test scripts and wrote the manuscript. M.R.W. and T.M.O. designed statistical models. D.S.L. extracted and prepared DNA for sequencing and managed OTU pipeline. S.G.T. and D.C. coordinated DNA amplification and sequencing. M.R.W., D.S.L., J.L.D. and T.M.O. contributed to manuscript revisions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Aikawa, S., Kobayashi, M.J., Satake, A., Shimizu, K.K. & Kudoh, H. (2010). Robust control of the seasonal expression of the Arabidopsis FLC gene in a fluctuating environment. *P. Natl. Acad. Sci. USA*, 107, 11632–11637.

Anderson, J.T., Lee, C.R. & Mitchell-Olds, T. (2011). Life-history QTLs and natural selection on flowering time in *Boechera stricta*, a perennial relative of Arabidopsis. *Evolution*, 65, 771–787.

Anderson, J.T., Wagner, M.R., Rushworth, C.A., Prasad, K.V.S.K. & Mitchell-Olds, T. (2014). The evolution of quantitative traits in complex environments. *Heredity*, 112, 4–12.

Batten, K.M., Scow, K.M. & Espeland, E.K. (2007). Soil Microbial Community Associated with an Invasive Grass Differentially Impacts Native Plant Performance. *Microbial Ecol.*, 55, 220–228.

Berendsen, R.L., Pieterse, C.M.J. & Bakker, P.A.H.M. (2012). The rhizosphere microbiome and plant health. *Trends Plant Sci.*, 17, 478–486.

Berg, G. & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.*, 68, 1–13.

Berns, A.E., Philipp, H., Narres, H.-D., Burauel, P., Vereecken, H. & Tappe, W. (2008). Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. *Eur. J. Soil Sci.*, 59, 540–550.

Borchert, R., Meyer, S., Felger, R. & Porter-Bolland, L. (2004). Environmental control of flowering periodicity in Costa Rican and Mexican tropical dry forests. *Glob. Ecol. Biogeog.*, 13, 409–425.

Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, M. *et al.* (2010). Linkage and Association Mapping of Arabidopsis thaliana Flowering Time in Nature. *PLoS Genet*., 6, e1000940.

Bradshaw, A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet*., 13, 115–155.

Brun, L.A., Le Corff, J. & Maillet, J. (2003). Effects of elevated soil copper on phenology, growth and reproduction of five ruderal plant species. *Environ. Pollut*., 122, 361–368.

Brys, R., Shefferson, R. & Jacquemyn, H. (2011). Impact of herbivory on flowering behaviour and life history trade-offs in a polycarpic herb: a 10-year experiment. *Oecologia*, 166, 293–303.

Bulgarelli, D., Rott, M., Schlaeppi, K., Loren, Ver, van Themaat, E., Ahmadinejad, N. *et al.* (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, 488, 91–95.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L. & Schulze-Lefert, P. (2013). Structure and Functions of the Bacterial Microbiota of Plants. *Annu. Rev. Plant Biol*., 64, 807–838.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K. *et al.* (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, 7, 335–336.

Castro, H.F., Classen, A.T., Austin, E.E., Norby, R.J. & Schadt, C.W. (2009). Soil Microbial Community Responses to Multiple Experimental Climate Change Drivers. *Appl. Environ. Microb*., 76, 999–1007.

Cleland, E.E., Chuine, I., Menzel, A., Mooney, H.A. & Schwartz, M.D. (2007). Shifting plant phenology in response to global change. *Trends Ecol. Evol*., 22, 357–365.

Crimmins, T., Crimmins, M. & Bertelsen, C. (2013). Spring and summer patterns in flowering onset, duration, and constancy across a water-limited gradient. *Am. J. Bot*., 100, 1137–1147.

Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods*, 10, 996–998.

Ettema, C.H. & Wardle, D.A. (2002). Spatial soil ecology. *Trends Ecol. Evol*., 17, 177–183.

Fierer, N. & Jackson, R.B. (2006). The diversity and biogeography of soil bacterial communities. *P. Natl. Acad. Sci. USA*, 103, 626–631.

Forrest, J. & Miller-Rushing, A.J. (2010). Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philos. T. Roy. Soc. B-Biol. Sci*., 365, 3101–3112.

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol*., 21, 394–407.

van der Heijden, M.G.A., Bardgett, R.D. & van Straalen, N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett*., 11, 296–310.

Hol, W.H.G., de Boer, W., Termorshuizen, A.J., Meyer, K.M., Schneider, J.H.M., van Dam, N.M. *et al.* (2010). Reduction of rare soil microbes modifies plant-herbivore interactions. *Ecol. Lett*., 13, 292–301.

Kembel, S.W., Wu, M., Eisen, J.A. & Green, J.L. (2012). Incorporating 16S Gene Copy Number Information Improves Estimates of Microbial Diversity and Abundance. *PLoS Comput. Biol*., 8, e1002743.

Korves, T. & Bergelson, J. (2003). A developmental response to pathogen infection in Arabidopsis. *Plant Physiol*., 133, 339–347.

Lau, J.A. & Lennon, J.T. (2011). Evolutionary ecology of plant-microbe interactions: soil microbial structure alters selection on plant traits. *New Phytol*., 192, 215–224.

Lau, J.A. & Lennon, J.T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. *P. Natl. Acad. Sci. USA*, 109, 14058–14062.

Lee, C.-R. & Mitchell-Olds, T. (2013). Complex trait divergence contributes to environmental niche differentiation in ecological speciation of *Boechera stricta*. *Mol. Ecol*., 22, 2204–2217.

Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S. *et al.* (2012). Defining the core Arabidopsis thaliana root microbiome. *Nature*, 488, 86–90.

Lundberg, D.S., Yourstone, S., Mieczkowski, P., Jones, C.D. & Dangl, J.L. (2013). Practical innovations for high-throughput amplicon sequencing. *Nat. Methods*, 10, 999–1002.

MacColl, A.D.C. (2011). The ecological causes of evolution. *Trends Ecol. Evol*., 26, 514–522.

Magoč, T. & Salzberg, S.L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27, 2957–2963.

Miner, B., Sultan, S., Morgan, S., Padilla, D. & Relyea, R. (2005). Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol*., 20, 685–692.

Oksanen, J., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R.B. *et al.* (2013). *vegan: Community Ecology Package*. R package version 2.0-9. Available at: http://CRAN.R-project.org/package=vegan. Last accessed 17 November 2013.

Pigliucci, M., Schlichting, C. & Whitton, J. (1995). Reaction norms of Arabidopsis. 2. Response to stress and unordered environmental variation. *Funct. Ecol*., 9, 537–547.

R Core Team. (2013). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/. Last accessed 29 September 2013.

Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J. & Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett*., 9, 981–993.

Rodríguez, H. & Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv*., 17, 319–339.

Rushworth, C.A., Song, B.-H., Lee, C.-R. & Mitchell-Olds, T. (2011). Boechera, a model system for ecological genomics. *Mol. Ecol*., 20, 4843–4857.

Ryser, P. & Sauder, W. (2006). Effects of heavy-metal-contaminated soil on growth, phenology and biomass turnover of Hieracium piloselloides. *Environ. Pollut*., 140, 52–61.

Song, B.H., Clauss, M.J., Pepper, A. & Mitchell-Olds, T. (2006). Geographic patterns of microsatellite variation in *Boechera stricta*, a close relative of Arabidopsis. *Mol. Ecol*., 15, 357–369.

Stanton, M.L., Roy, B.A. & Thiede, D.A. (2000). Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution*, 54, 93–111.

Strauss, S.Y. & Whittall, J.B. (2006). Non-pollinator agents of selection on floral traits. In *Ecology and Evolution of Flowers*. (eds Harder, L.D., Barrett, S.C.H.). Oxford University Press Inc, New York, pp. 120–138.

Thompson, J.D. (1991). Phenotypic plasticity as a component of evolutionary change. *Trends Ecol. Evol*., 6, 246–249.

Van Zandt, P.A. & Mopper, S. (2002). Delayed and carryover effects of salinity on flowering in Iris hexagona (Iridaceae). *Am. J. Bot*., 89, 1847–1851.

Vorholt, J.A. (2012). Microbial life in the phyllosphere. *Nat. Rev. Microbiol*., 10, 828–840.

Wade, M. & Kalisz, S. (1990). The causes of natural selection. *Evolution*, 44, 1947–1955.

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*. Springer, New York.

SUPPORTING INFORMATION

Additional Supporting Information may be downloaded via the online version of this article at Wiley Online Library (www.ecologyletters.com).

Editor, John Klironomos
Manuscript received 7 January 2014
First decision made 9 February 2014
Manuscript accepted 28 February 2014

Appendix S1. Descriptions of natural habitats used in this study.

Clockwise, from top left:

Jackass Meadow (JAM): high-altitude meadow on edge of pine forest (photo: J. Lipkowitz)

Mahogany Valley (MAH): meadow between pine forest and sagebrush scrub (photo: J. Lipkowitz)

Parker Meadow (PAR): high-altitude meadow in dead pine forest (burned 2007) (photo: M. Wagner)

Silver Creek (SIL): lush, low-altitude pine forest adjacent to creek (photo: T. Mitchell-Olds)

Site	Abbreviation	Elevation (m)	Latitude (°N)	Longitude (°W)
Silver Creek	SIL	1812	44.90	114.40
Parker Meadow	PAR	2671	44.61	114.52
Mahogany Valley	MAH	2531	44.18	113.74
Jackass Meadow	JAM	2676	44.97	114.08

Appendix S2. Detailed methods.

Soil sterilization and greenhouse experiment setup.
Excesses of Fafard 4P (Conrad Fafard Inc., Agawam, MA, USA) and Metromix 200 (Sun Gro Horticulture Inc., Vancouver, BC, Canada) potting soils were autoclaved twice for 90 min at 121°C, separated by a 24 h cooling period at room temperature. We loosely packed sterile Fafard 4P in the bottom three inches of 49-mL single-cell pots (Ray Leach Cone-tainers, Stuewe & Sons Inc., Tangent, OR, USA), and sterile Metromix 200 in the top inch. Pots were pre-washed with dish soap and SA-20 disinfectant (Southern Agricultural Insecticides Inc., Hendersonville, NC, USA). Field soils also were autoclaved twice for 90 min at 121°C, with a 24 h cooling period (at room temperature) between sterilizations, mixed with approximately ¼ volume of autoclaved perlite to improve drainage, and packed loosely into the same 49-mL single-cell pots.

Surface sterilization of seeds.
Seeds were surface sterilized using five washes: (1) vortexed one minute and inverted in 70% ethanol with 0.1% Triton X-100, (2) vortexed, inverted, and soaked 15 minutes in 10% bleach with 0.1% Triton X-100; (3-5) rinsed 3 times with sterile diH_2O to remove bleach.

Climate control for three stages of experiment.
Seeds were germinated for one week in a growth chamber at 22°C under ambient relative humidity and 11-hour days. Plants were grown to adulthood in the greenhouse with 16-hour days, photosynthetically active radiation maintained between 600 and 2000 μmol/sec/cm^2, daytime temperatures between 65°F and 70°F, nighttime temperatures between 55°F and 60°F, and relative humidity between 37% and 52%. Vernalization took place in a growth chamber at 4°C with 11-hour days and ambient relative humidity. After vernalization plants were allowed to flower and set fruit in the greenhouse with the same conditions as above.

Justification of linear mixed model.
We included three growth-related covariates in our models of flowering time and selection (Tables 1 and 2): elongation rate (mm/day), height at first flowering (mm), and number of leaves per mm stem. We acknowledge that in nature, the emergent phenotype (i.e. simply the observed speed to flowering, which has been influenced by environmental factors affecting growth) has great ecological and evolutionary relevance; for this reason we discuss both selection gradients and selection differentials on flowering time, and provide results from models without covariates in Supplementary tables. However, our question of whether soil microbiota affect flowering time *per se* is best answered by controlling for their effects on growth (which are already appreciated; van der Heijden et al. 2008), and therefore in the main text we report effect estimates from the original models including the covariates.

Appendix S3. A method to search for microbial community members that affect a phenotype of interest.

Problem: An experiment showed that qualitatively different environmental microbiomes alter a phenotype of interest, but the ultimate goal is to find specific organisms associated with the phenotypic effect.

Approach:

1. **Sample environments for DNA extraction and 16S analysis**. Ideally, each phenotyped individual would have its own 16S community measurement. Other possibilities include block-level sampling or (as in our experiment, see main text) one aggregate sample per microbiome treatment.
2. **Principal coordinate analysis** provides a quantitative summary of the microbiome; each individual PCo describes a different component of the community measured in each sample.
3. **For a more focused test, identify candidate PCo(s)**. In our case, we chose the PCo that separated the treatments that caused the phenotypic extremes in our experiment, and ignored the others.
4. **Test for a correlation between the candidate PCo(s) and the phenotype**. If you measured the microbiome for each individual, the PCo value can be substituted directly into a full linear model where the qualitative "Treatment" would have been: e.g. PHENOTYPE = SUBSPECIES + TREATMENT + SUBSPECIES × TREATMENT would become PHENOTYPE = SUBSPECIES + PCO1 + PCO2 + SUBSPECIES × PCO1 + SUBSPECIES × PCO2 . If you pooled microbiome samples, e.g. one microbiome measured per block, regress the mean phenotype in each block onto the block's values of candidate PCo(s). To account for individual-level covariates (e.g. genotype), use phenotype residuals as the response variable.
5. **Identify OTUs with strong associations to the candidate PCo**. Like each environmental sample, each OTU has a score or loading on each PCo. Choose a subset of OTUs with high loadings on your most promising candidate PCo.
6. **Test for a correlation between the candidate OTU(s) and the phenotype**. See step #4 above: the abundance of the candidate OTU is analogous to the candidate PCo score. Note, however, that unlike PCoA axes, OTUs are likely to be multicollinear and therefore may need to be tested one at a time in separate models; significance should then be corrected for multiple comparisons.
7. **To search for broader taxa associated with the phenotype**, compare relative abundances of phyla, classes, orders, or families between environmental samples associated with phenotypic extremes. Alternatively or in addition, test for a correlation between the phenotype and relative abundance of a candidate taxon (analogous to steps #4 and #6, above).

Table S1 List of *Boechera stricta* genotypes, collection locations, and subspecies

Genotype	Abbrev	Elev (m)	Lat (°N)	Lon (°W)	Subsp
Alder Creek	ALD	2130	44.806650	114.270633	W
Alder Creek Upper	ALU	2174	44.803733	114.276483	W
Bayhorse Meadow	BHM	2465	44.406000	114.381150	W
Bayhorse Saddle	BHS	2650	44.410000	114.407733	E
Bannock	BNK	2493	44.791000	113.312550	E
Bearskin Creek	BSC	1965	44.416000	115.470300	W
Bear Valley Creek	BVC	1948	44.411000	115.372000	W
Bear Valley Meadow	BVM	2087	44.795000	113.782150	W
Coiner Prairie	COI	2003	45.156395	114.151722	W
Cold Canyon	COL	2373	43.839000	114.294667	E
Deep Creek Ridge	DCR	2123	45.118533	114.158433	W
Deadwood	DDW	2028	44.296000	115.479867	W
Double Springs Pass North	DSP	2582	44.231633	113.838117	E
Eagle Mountain	EAG	2210	45.541000	113.826533	E
East Creek Middle Fork	ECM	2647	44.536000	112.617967	E
Elkhorn	ELK	1975	46.268000	111.927467	W
Floodplain Forest	FPF	2138	44.797000	113.798217	E
Gibbons Pass Road	GIB	1960	45.679000	113.832767	W
Grand Prize Trailhead	GPT	2351	43.937000	114.690367	E
Humphrey Crest	HUM	2156	44.523000	112.192450	E
Iron Flats	IRF	1880	44.941000	114.121100	W
Jackass Meadow	JAM	2691	44.966883	114.085250	E
Jordan Creek Upper	JCU	2333	44.457000	114.752883	E
Lost Trail Meadow	LTM	2462	45.705182	113.988730	W
Mahogany Camp	MAH	2526	44.182000	113.739383	E
Middle Fork Peak	MFP	2758	44.963000	114.655767	E
Mill Creek	MIL	2259	44.367000	113.356783	E
Mono Creek	MON	2112	45.534000	113.080017	W
Moonrise Ridge	MRR	2673	44.642000	114.528967	E
Napoleon Hill	NAP	2282	45.336000	114.003917	W
Pass Creek South	PCS	2319	44.027000	113.452950	E
Pintler Creek	PIN	1933	45.853000	113.438500	E
Pioneer Creek	PIO	2040	45.551000	113.774233	W
Parker Meadow ‘A’	PMA	2681	44.616000	114.518433	E
Parker Meadow ‘B’	PMB	2681	44.616000	114.518433	E
Ruby Creek	RUB	2026	45.546700	113.763200	W
Sagebrush Meadow	SBM	2444	44.426000	112.894467	E
Seafoam	SEA	2053	44.444000	115.089333	W
Silver Creek Upper	SIL	1843	44.912000	114.386600	W
Sleeping Deer	SLD	2848	44.754000	114.679383	E
Taylor River	SAD	2517	38.707000	106.804483	E
Thatcher	THA	2017	44.366000	115.143483	W
Twelve Mile	TWM	2071	44.941000	113.847400	E
Twin Saddle	TWS	2834	44.622000	114.496833	E
Van Horn	VAN	2014	44.406000	115.285750	W
Whiskey Creek	WHC	2061	44.569783	115.541883	W
Whitehawk	WHH	2027	44.288000	115.481333	W
Yellowjacket	YWJ	1794	44.965000	114.602050	W

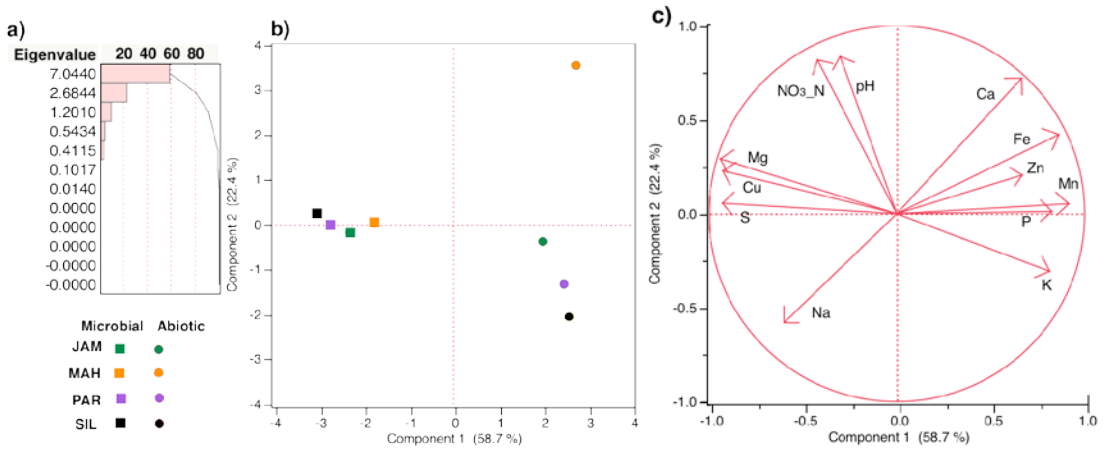

Figure S1 Principal components analysis of correlations between 12 physical and chemical properties of the eight soil treatments. "Microbial" samples consisted of sterilized potting soil inoculated with soil microbiota extracted from one of four natural soils; "abiotic" samples were autoclaved natural soils. Unplanted soil controls (8 per treatment) were collected the day after the end of the experiment, pooled, and submitted for analysis at the Texas A&M Soil, Water, and Forage Testing Laboratory (College Station, TX, USA). (a) Summary plot showing partitioning of soil variation into twelve axes. (b) Principal components plot showing separation of soil treatments along two major axes of variation, which explain 58.7% (horizontal axis) and 22.4% (vertical axis) of total variation respectively. (c) Contributions of 12 soil properties to the first two principal components

Table S2 Chemical and physical properties of soil treatments at end of experiment. Unplanted soil controls (8 per treatment) were collected the day after the end of the experiment, pooled, and submitted for analysis at the Texas A&M Soil, Water, and Forage Testing Laboratory (College Station, TX, USA). All units are *ppm* with the exceptions of pH (unitless). Due to the small volume of treated soils available for nutrient analysis, we could not perform the replicate tests needed to statistically test for differences between soils.

(a) Potting soil inoculated with soil microbiota

	pH	NO_3-N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
JAM	6.8	37	16	79	1096	575	174	26	12.2	0.5	8.5	0.79
MAH	6.9	29	15	73	1027	547	114	20	10.3	0.6	9.8	0.59
PAR	6.8	51	16	67	940	543	170	29	13.5	0.6	7.9	1.00
SIL	6.8	47	13	70	1130	561	298	27	13.2	0.5	8.8	1.06
var	.002	99	2	26	7044	212	6031	15	2.1	0.003	0.63	0.05

(b) Sterilized field soils

	pH	NO_3-N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
JAM	6.8	36	76	535	1350	173	10	21	22.6	0.6	112.6	0.11
MAH	7	57	69	285	2402	306	13	11	41.6	5.1	108.6	0.41
PAR	6.5	12	108	251	1407	98	9	16	29.8	1.1	55.0	0.20
SIL	6.6	8	36	633	1257	134	8	28	24.7	5.6	119.3	0.17

var	.05	520	872	35078	286846	8248	5	53	72.3	6.8	875.0	0.02

Table S3 Parameter estimates and standard errors for mixed models of flowering time (FLT) and fruit number (FRN) in response to biotic and abiotic soil treatments. Reported values are least square means unless otherwise noted. Flowering time units are days after vernalization; height units are mm; fecundity measured as number of fruits

Model:	FLT_{biotic} (Table 1a)		$FLT_{abiotic}$ (Table 1b)		FRN_{biotic} (Table 2a)		$FRN_{abiotic}$ (Table 2b)	
Term:	Est.	StdEr	Est.	StdEr	Est.	StdEr	Est.	StdEr
Intercept	31.91	1.45	30.84	1.34	0.504	0.61	2.350	0.86
Subspecies: E	34.22	0.80	34.92	0.56	2.162	0.17	1.837	0.20
Subspecies: W	37.50	0.87	35.16	0.64	2.917	0.19	3.110	0.23
Treatment: JAM	34.83	0.68	35.75	0.61	2.558	0.15	2.702	0.24
Treatment: MAH	36.98	0.67	33.74	0.59	2.329	0.15	3.200	0.22
Treatment: PAR	35.57	0.69	37.14	0.95	2.728	0.15	1.362	0.38
Treatment: SIL	36.07	0.66	33.52	0.59	2.543	0.14	2.630	0.23
T×S: JAM×E	34.05	0.95	35.91	0.90	2.122	0.22	2.132	0.37
T×S: MAH×E	34.55	0.93	33.61	0.78	2.217	0.21	2.259	0.30
T×S: PAR×E	34.00	0.99	37.04	1.12	2.295	0.24	1.146	0.44
T×S: SIL×E	34.29	0.94	33.10	0.78	2.015	0.22	1.812	0.32
T×S: JAM×W	35.61	1.06	35.60	0.82	2.994	0.24	3.273	0.32
T×S: MAH×W	39.41	1.07	33.87	0.89	2.441	0.26	4.141	0.35
T×S: PAR×W	37.14	1.03	37.25	1.51	3.161	0.23	1.578	0.61
T×S: SIL×W	37.85	1.01	33.93	0.86	3.070	0.23	3.448	0.33
Genotype(Subsp) *	34.50	8.97	5.654	4.99	20.38	6.17	2.594	5.06
Geno(Ssp) × T *	0.000	4.23	4.720	7.60	0.000	4.03	19.38	8.64
Block *	0.340	0.93	2.170	2.49	0.052	0.80	0.705	1.80
Flowering time †					0.001	0.02	-0.013	0.02
JAM × fl. time †					0.013	0.01	-0.043	0.02
MAH × fl. time †					0.006	0.01	0.031	0.02
PAR × fl. time †					0.026	0.01	0.006	0.03
SIL × fl. time †					-0.045	0.01	0.006	0.02
Elongation rate †	-11.14	0.64	-17.62	0.95	0.107	0.38	0.246	0.58
Hgt at flowering †	0.365	0.02	0.579	0.03	0.022	0.01	0.007	0.02
Leaves/mm stem †	4.425	1.11	5.346	1.16	1.156	0.30	-0.152	0.48

* Random effect; reported values are percent variation explained

† Continuous effect; reported values are linear coefficients

Table S4 Statistics for REML mixed models of flowering time for (a) soil microbial community treatments and (b) sterilized field soil treatments, without controlling for growth-related covariates. All effects are fixed except for Block, Genotype(Subspecies), and Genotype(Subsp.) × Treatment. For fixed effects the test statistic *F* is reported. For random effects, the test statistic χ^2 is reported, calculated as twice the difference between log likelihoods of the full model and the model with the random factor excluded. Compare with statistics for model including growth-related covariates, Table 1

	(a) Soil microbial communities (N = 451, Adj. R^2=0.60)			(b) Sterilized field soils (N = 336, Adj. R^2=0.43)		
	d.f.	F or χ^2	P	d.f.	F or χ^2	P
Treatment	3,101	3.5301	**0.0176**	3,74	8.8807	**<0.0001**
Subspecies	1,44	28.997	**<0.0001**	1,47	18.251	**<0.0001**
Treatment × Subspecies	3,102	2.3039	0.0814	3,74	1.4615	0.2320
Genotype (Subspecies)	2	72.383	**<0.00001**	2	23.181	**<0.0001**
Geno.(Ssp.) × Treatment	6	0.2579	>0.05	6	0	1
Block	1	0.4299	>0.05	1	0.4352	>0.05

Bold type indicates significance of at least P<0.05

Table S5 Statistics for REML mixed models of reproductive fitness for (a) soil microbial community treatments and (b) sterilized field soil treatments, without controlling for selection on growth-related covariates. All effects are fixed except for Block, Genotype(Subspecies), and Genotype(Subsp.) × Treatment. For fixed effects the test statistic *F* is reported. For random effects, the reported test statistic is χ^2, calculated as twice the difference between log likelihoods of the full model and the model with the random factor excluded. Note that in this model "flowering time" reflects the selection differential, i.e. both direct selection on flowering time and indirect selection on correlated traits. Compare with statistics for selection gradient on flowering time, Table 2

	(a) Soil microbial communities (N = 439, Adj. R^2=0.26)			(b) Sterilized field soils (N = 331, Adj. R^2=0.48)		
	d.f.	F or χ^2	P	d.f.	F or χ^2	P
Treatment	3,83	1.3281	0.2708	3,100	6.5986	**0.0004**
Subspecies	1,48	6.5373	**0.0138**	1,58	7.5059	**0.0082**
Flowering time	1,405	0.1111	0.7391	1,313	6.4731	**0.0114**
Treatment × Subspecies	3,93	1.4695	0.2280	3,105	0.8088	0.4917
Treatment × Fl. time	3,249	2.8348	**0.0388**	3,301	1.6838	0.1705
Genotype (Subspecies)	2	43.601	< **0.0001**	2	4.0324	>0.05
Geno.(Ssp.) × Treatment	6	0	1	6	5.9867	>0.05
Block	1	0	1	1	0.3349	>0.05

Bold type indicates significance of at least P<0.05

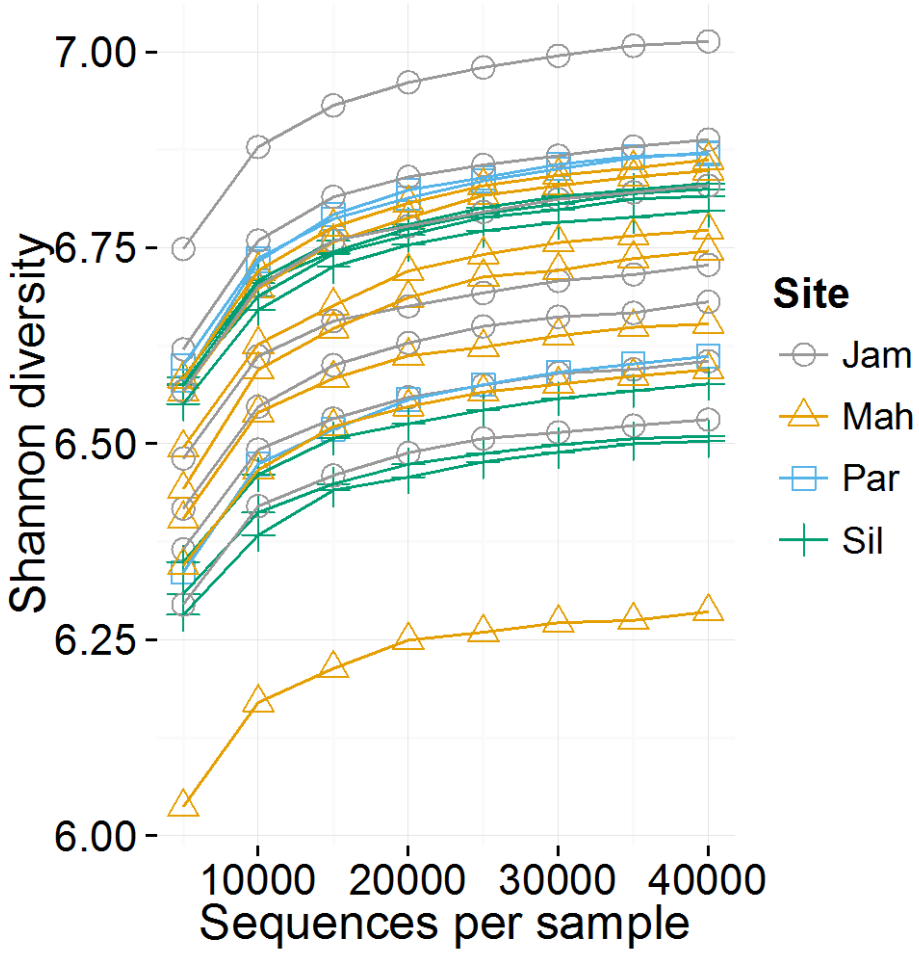

Figure S2 Rarefaction curves to 40,000 sequences (16S rDNA sequences that could be assigned to a reproducible OTU; see main text for details) for all 24 field soil samples. Alpha diversity was estimated using Shannon's diversity index.

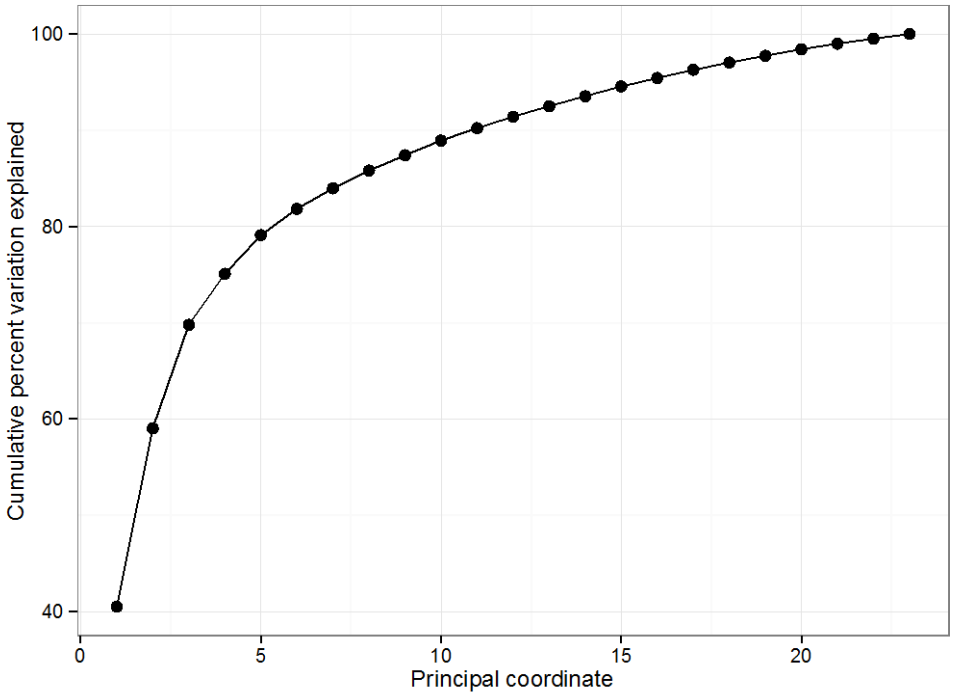

Figure S3 Scree plot of microbiome principal coordinates analysis

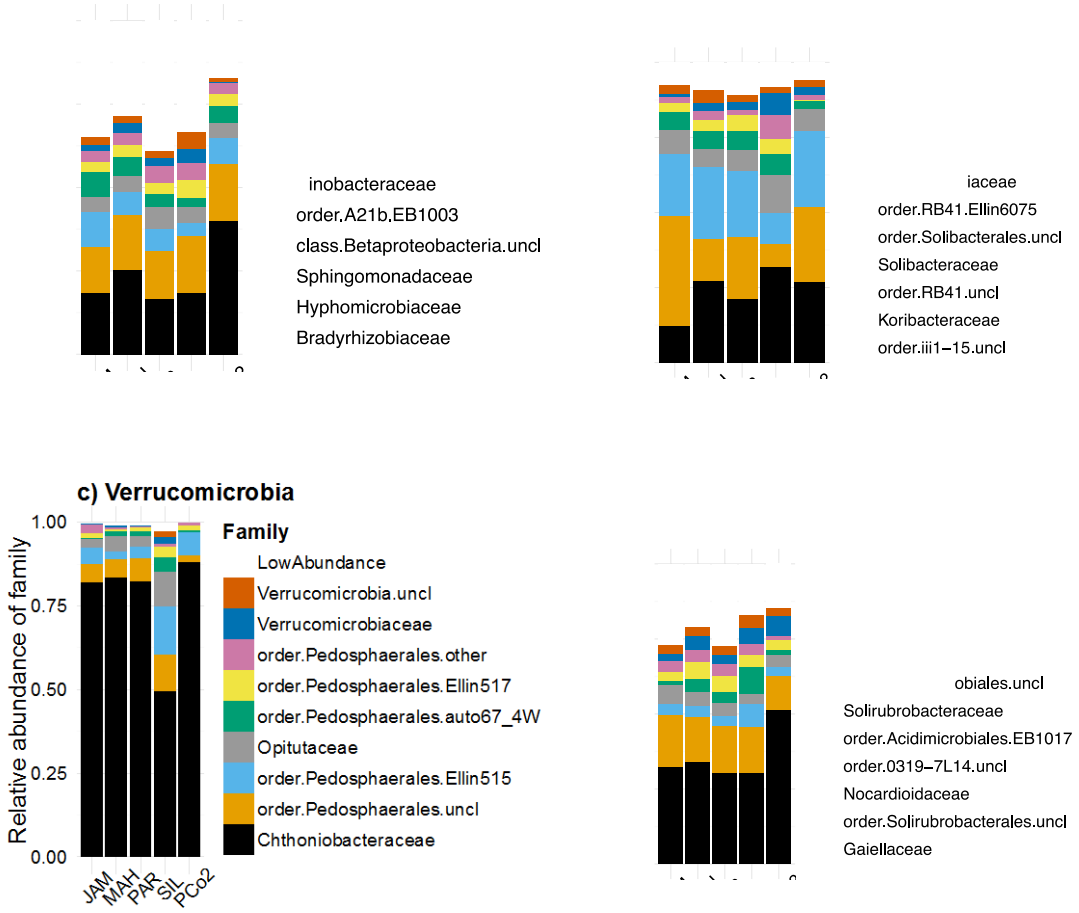

Figure S4 Relative abundances of the dominant families within the four most abundant phyla (a) Proteobacteria, (b) Acidobacteria, (c) Verrucomicrobia, and (d) Actinobacteria in four natural soil communities (pooled samples within sites) and in the top 5% of OTUs most highly correlated with PCo2, a putative predictor of flowering time. All remaining families were pooled into the category “Low abundance.” Raw OTU abundances were corrected for 16S gene copy number variation.

Table S6 The ten OTUs with highest correlations with PCo2; their correlations with PCo2; their taxonomic designation at lowest level of classification; estimates of their direct effect on flowering time; *P* values of direct effects and Benjamini-Hochberg false discovery rate-corrected *P* values. Estimates and statistics are from a linear regression of mean flowering time residuals of western genotypes in each treatment after controlling for block effects, genetic differences, and continuous covariates elongation rate (mm/day), height at first flowering (mm), and leaves per mm stem. The units of the parameter estimates are days per OTU representative in a community rarefied to 40,000 sequences, corrected for 16S gene copy number variation

	PCo2 Score	Taxonomic designation	Est. (days/ind.)	*P*	P_{FDR}
OTU211	0.604	Chthoniobacteraceae	-0.00007	0.99	0.99
OTU240	0.335	Gaiellaceae	0.0028	0.74	0.88
OTU558	0.206	Chloracidobacteria order RB41	0.0035	0.79	0.88
OTU744	0.155	Chloroflexi class Gitt-GS-136	0.0201	0.31	0.53
OTU1735	0.147	Bacillales	0.0732	0.22	0.53
OTU35562	0.153	Gemmatimonadetes N1423WL	0.0097	0.32	0.53
OTU47051	0.405	Chloroflexi Ellin6529	0.0092	0.18	0.53
OTU86905	0.145	Bacillaceae	0.0877	0.13	0.53
OTU96997	0.224	Hyphomicrobiaceae	0.0182	**0.037**	0.37
OTU99474	-0.356	Chthoniobacteraceae	-0.0024	0.39	0.57

Bold type indicates significance of at least $P<0.05$

Table S7 Comparison of copy number-adjusted relative abundances of major families in the four dominant phyla between slow-flowering MAH and late-flowering JAM soils. Significance of enrichment/depletion was determined by Wilcoxon Rank Sum tests and adjusted using the Benjamini-Hochberg false discovery rate

Phylum	Family	JAM	MAH	P_{FDR}
Proteobacteria	Bradyrhizobiaceae	9.3%	13.0%	0.053
Proteobacteria	Hyphomicrobiaceae	14.2%	16.7%	0.33
Proteobacteria	Sphingomonadaceae	9.9%	6.9%	0.28
Proteobacteria	Betaproteobacteria.uncl	4.7%	4.9%	0.86
Proteobacteria	Order.A21b.EB1003	7.7%	5.5%	0.48
Proteobacteria	Sinobacteraceae	3.1%	3.6%	0.33
Proteobacteria	Order.SC-I-84.uncl	3.0%	3.6%	0.48
Proteobacteria	Myxococcales.uncl	2.0%	2.9%	**0.040**
Proteobacteria	Order.Ellin329.uncl	2.2%	2.3%	1
Acidobacteria	Order.iii1-15.uncl	6.7%	14.4%	0.20
Acidobacteria	Koribacteraceae	36.3%	13.6%	**0.004**
Acidobacteria	Order.RB41.uncl	20.7%	24.1%	0.10
Acidobacteria	Solibacteraceae	80%	5.8%	**0.040**
Acidobacteria	Solibacterales.uncl	6.0%	6.4%	0.38
Acidobacteria	Order.RB41.Ellin6075	3.1%	3.5%	0.56
Acidobacteria	Acidobacteriaceae	2.0%	3.0%	0.62
Acidobacteria	Acidobacteria-5.uncl	1.1%	2.6%	**0.004**
Acidobacteria	Order.Ellin6513.uncl	3.0%	4.5%	0.08
Verrucomicrobia	Chthoniobacteraceae	81.4%	83.4%	0.70
Verrucomicrobia	Pedosphaerales.uncl	5.7%	5.6%	0.62
Verrucomicrobia	Pedosphaerales.Ellin515	4.9%	2.3%	**0.005**
Verrucomicrobia	Opitutaceae	2.9%	4.7%	**0.040**
Verrucomicrobia	Pedosphaerales.auto67_4W	0.25%	1.3%	**0.004**
Verrucomicrobia	Pedosphaerales.Ellin517	1.5%	0.64%	**0.031**
Verrucomicrobia	Pedosphaerales.other	2.5%	0.50%	**0.004**
Verrucomicrobia	Verrucomicrobiaceae	0.27%	0.64%	**0.031**
Verrucomicrobia	Verrucomicrobia.other	0.03%	0.15%	**0.005**
Actinobacteria	Gaiellaceae	16.8%	17.5%	0.62
Actinobacteria	Solirubrobacterales.uncl	17.3%	14.7%	**0.040**
Actinobacteria	Nocardioidaceae	3.5%	4.0%	0.62
Actinobacteria	Order.0319-7L14.uncl	6.3%	4.3%	0.10
Actinobacteria	Acidimicrobiales.EB1017	1.4%	4.6%	**0.004**
Actinobacteria	Solirubrobacteraceae	2.9%	5.6%	**0.004**
Actinobacteria	Acidimicrobiales.uncl	3.8%	4.0%	0.38
Actinobacteria	Mycobacteriaceae	2.3%	3.1%	**0.031**
Actinobacteria	Microbacteriaceae	3.0%	3.1%	0.86

Bold type indicates significance of at least $P<0.05$