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New Phytologist

Research

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Received: *26 December 2023*
Accepted: *7 May 2024*

New Phytologist (2024)
doi: 10.1111/nph.19870

Key words: denitrification inhibition and stimulation, intraspecific variation, niche construction, nitrification inhibition and stimulation, nitrogen cycle, plant–soil feedback.

Summary

- In natural systems, different plant species have been shown to modulate specific nitrogen (N) cycling processes so as to meet their N demand, thereby potentially influencing their own niche. This phenomenon might go beyond plant interactions with symbiotic microorganisms and affect the much less explored plant interactions with free-living microorganisms involved in soil N cycling, such as nitrifiers and denitrifiers.
- Here, we investigated variability in the modulation of soil nitrifying and denitrifying enzyme activities (NEA and DEA, respectively), and their ratio (NEA : DEA), across 193 *Arabidopsis thaliana* accessions. We studied the genetic and environmental determinants of such plant–soil interactions, and effects on plant biomass production in the next generation.
- We found that NEA, DEA, and NEA : DEA varied *c.* 30-, 15- and 60-fold, respectively, among *A. thaliana* genotypes and were related to genes linked with stress response, flowering, and nitrate nutrition, as well as to soil parameters at the geographic origin of the analysed genotypes. Moreover, plant-mediated N cycling activities correlated with the aboveground biomass of next-generation plants in home vs away nonautoclaved soil, suggesting a transgenerational impact of soil biotic conditioning on plant performance.
- Altogether, these findings suggest that nutrient-based plant niche construction may be much more widespread than previously thought.

Introduction

Though terrestrial plants can be perceived as constrained by environmental factors due to their sessile condition, they actually have a remarkable capacity to modulate local biotic and abiotic conditions. Such ability is, for instance, deployed to guarantee the acquisition of major limiting nutrients to plant growth, such as nitrogen (N). Under N limitation, plants can adopt different strategies for N acquisition that involve (1) root development; (2) plant affinity for soil N forms; and (3) the modification of rhizospheric abiotic conditions (e.g. pH, oxygen availability, and chemical profile due to root exudation), which can entail local biotic changes that, in turn, impact nutrient cycling (Richardson *et al.*, 2009; Moreau *et al.*, 2019; Pantigoso *et al.*, 2022). In natural systems, the latter plant strategy may be particularly common because N often occurs in forms and states that make it unavailable to plants, a condition that promotes plant dependency on specific microorganisms capable of mobilizing and transforming N in the soil (Fontaine *et al.*, 2024). When plant dependency on soil microbes is strong, it can lead to symbiotic relationships, such as the well-studied plant interactions with rhizobium for atmospheric N_2 fixation or with mycorrhizal fungi (Heath & Grillo, 2016; Petipas *et al.*, 2021; Magnoli & Bever, 2023). Beyond the tight host-symbiont associations, plants can also interact with free-living microorganisms belonging to functional groups (or guilds) that transform N in the soil (Fernandez *et al.*, 2022).

Key microbe-mediated N transformations include mineralization, nitrification, and denitrification. Those correspond to a chain of chemical reactions that respectively produce ammonium (NH_4^+), nitrate (NO_3^-), and gaseous N forms such as nitrous oxide or dinitrogen. NH_4^+ and NO_3^- are major N forms assimilated by plants, and their availability to these organisms depends on nitrification, which produces NO_3^- from NH_4^+, and

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denitrification, which promotes NO_3^- reduction (Jackson *et al.*, 2008). For instance, high nitrification and low denitrification in soil make NO_3^- more available to plants, while low nitrification and high denitrification decrease NO_3^- availability. In the second case, NH_4^+ availability may increase, favouring plants that can efficiently assimilate NH_4^+ (Boudsocq *et al.*, 2012; Lata *et al.*, 2022). It can be hence advantageous for plants to modulate nitrification and/or denitrification and influence the NH_4^+-to-NO_3^- balance determined by these processes in order to better meet their mineral N requirements.

Plant modulation of soil nitrification and denitrification is a widespread phenomenon (Wheatley *et al.*, 1990; Crush, 1998; Patra *et al.*, 2006; Bardon *et al.*, 2018) that occurs through different mechanisms. Plants can compete with (de)nitrifiers for N forms (e.g. plant-nitrifiers competition for NH_4^+), influence soil environmental conditions that affect (de)nitrifiers (such as pH; Richardson *et al.*, 2009; Moreau *et al.*, 2019; Pantigoso *et al.*, 2022), or directly stimulate or inhibit nitrification and/or denitrification through biological (plant-mediated) nitrification inhibition (BNI; Lata *et al.*, 2022) and denitrification inhibition (BDI; Bardon *et al.*, 2014). Since intraspecific variation in the influence of plants on nitrification and denitrification exists, an evolutionary role has been suggested for this capacity (Lata *et al.*, 2022). For instance, Lata *et al.* (2004) showed that two populations of the same tropical grass species had different impacts on soil nitrification. The regulation of N processes by distinct genotypes of a single species has also been demonstrated, notably for *Arabidopsis thaliana* (Lu *et al.*, 2018), grasses (Bowatte *et al.*, 2016; Teutscherová *et al.*, 2022), sorghum (Tesfamariam *et al.*, 2014), rice (S. Chen *et al.*, 2022; Zhang *et al.*, 2022), maize (Mwafulirwa *et al.*, 2021), and wheat (Dubs *et al.*, 2023). However, the extent of the intraspecific variation underlying plant influence on nitrification and denitrification and its impact on evolution remain largely unknown (Lata *et al.*, 2022).

Organisms that modify environmental conditions can change the selective pressures acting on themselves and on other organisms, a phenomenon known as niche construction (Odling-Smee, 1988; Odling-Smee *et al.*, 2003, 2013). The niche construction concept is based on the principle of the existence of feedbacks between ecological and evolutionary processes (Odling-Smee *et al.*, 2003), which can both occur at contemporary time scales and promote short-term evolution (Laland *et al.*, 1999; Post & Palkovacs, 2009). However, demonstrating niche construction is challenging because it depends not only on controlling confounding sources of environmental variation, but also on showing three main conditions for selective processes to act within a population: (1) variation in the way organisms modify the environment, (2) heritability of the niche-constructing trait(s), (3) fitness impacts caused by the niche-constructing trait(s) (Odling-Smee *et al.*, 2013). In plants, these three conditions have been mostly investigated in a single study system in the context of intraspecific plant–soil feedback (PSF; Wagg *et al.*, 2015; Schweitzer *et al.*, 2018; Kirchhoff *et al.*, 2019; Gundale & Kardol, 2021).

Plant–soil feedback is a concept originally developed within the field of plant community ecology (Bever, 1994; Bever *et al.*, 1997) and is defined as plant impacts on soil biotic and abiotic properties ('soil conditioning') leading to consequences for plant performance (Bever *et al.*, 1997; Van der Putten *et al.*, 2013). It is often assessed through relative plant performance in home vs away soil, which is either sterilized or not in order to disentangle biotic (e.g. rhizobacteria favourable to plant growth) and abiotic (e.g. soil chemical parameters) soil effects (Brinkman *et al.*, 2010; Gundale & Kardol, 2021). The evolutionary consequences of PSF have been investigated only more recently through analyses at the genotype level (intraspecific PSF) and across generations (Gundale & Kardol, 2021). For instance, a recent study with *Populus angustifolia* showed that the local adaptation of this species was linked with N cycling processes that depended both on plant genetic factors and soil microbial communities (Van Nuland *et al.*, 2019). Despite such advances, our understanding of the mechanisms responsible for PSF is still limited, to the extent that this feedback is often referred to as a 'black box' (Kardol *et al.*, 2015; Abbott *et al.*, 2021). A valuable way to peek into such 'black box' is through the transgenerational study of the plant influence on specific groups of microorganisms impacting plant nutrition. Traditionally, this has been mostly performed in the context of plant associations with symbiotic microorganisms, such as N_2-fixing bacteria (e.g. Heath, 2010; Epstein *et al.*, 2023) and mycorrhizal fungi (e.g. Johnson *et al.*, 2010; Rekret & Maherali, 2019). Much less is known when it comes to nonsymbiotic associations that affect plant nutrition, particularly plant interactions with soil microbial functional groups involved in N cycling, like nitrifiers and denitrifiers (Fitzpatrick *et al.*, 2020; Fernandez *et al.*, 2022).

Arabidopsis thaliana is a model species in genetics that has been increasingly used to test ecological hypotheses (Weigel, 2012; Takou *et al.*, 2019). It is distributed across wide climatic gradients, and previous studies have demonstrated its local adaptation to climate (Méndez-Vigo *et al.*, 2011; Exposito-Alonso *et al.*, 2018) and soil parameters (Postma & Ågren, 2022). *Arabidopsis thaliana* is not colonized by mycorrhizal fungi but affects the endorhizosphere microbiome in comparable ways to other herbaceous species (Schneijderberg *et al.*, 2020). Moreover, distinct *A. thaliana* genotypes can recruit different bacteria in their rhizosphere (Micallef *et al.*, 2009; Lundberg *et al.*, 2012; Bergelson *et al.*, 2019; Kudjordjie *et al.*, 2021), and how they differentially condition the soil affects their performance (Bukowski & Petermann, 2014). All these facts and the availability of many completely re-sequenced ecotypes from across the globe (The 1001 Genomes Consortium, 2016) make *A. thaliana* a useful model for investigating intraspecific variation in plant influence on nitrification and denitrification, as well as the transgenerational implications of these plant–soil interactions.

Here, we investigated the influence of 193 genotypes of *A. thaliana* on nitrifying and denitrifying enzyme activities (NEA and DEA, respectively) in their rhizosphere. Accessions originating from across *A. thaliana*'s distribution range were grown in a common garden. We first assessed intraspecific variation and the genetic determinants underlying plant modulation of soil NEA, DEA, and the NEA : DEA ratio. Next, we investigated if these plant–soil interactions related to soil parameters at the geographic

origin of the analysed *A. thaliana* accessions (hereafter soil geographic parameters) in a potentially adaptive way. Finally, we tested for transgenerational effects of soil biotic conditioning with a subset of 20 *A. thaliana* genotypes contrasting in terms of their influence on NEA : DEA by assessing plant biomass production in home vs away soil (intraspecific PSF) in next-generation plants. We specifically addressed the following questions: (1) What is the extent of the intraspecific variation regarding plant influence on soil NEA, DEA, and NEA : DEA ratio in *A. thaliana*?; (2) How genetically determined is such variation and which genes may be involved?; (3) Do soil geographic parameters explain variation in the influence of *A. thaliana* genotypes on soil NEA, DEA, and NEA : DEA?; and (4) Does this plant-mediated soil impact produce transgenerational effects on *A. thaliana*'s biomass production?

Materials and Methods

Common garden experiment and soil sampling

We performed a completely randomized outdoor common garden experiment between February and July 2021 in the experimental field of Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), Montpellier, France (43°38′19″N, 3°51′44″E; Fig. 1a). We placed seeds of 193 accessions of *Arabidopsis thaliana* (L.) Heynh. (Fig. 1a) in pots of 0.08 l filled with a steam-sterilized soil mixture composed of 50% river sand, 37.5% calcareous clay soil from the experimental field at CEFE, and 12.5% blond peat moss. The aim of soil steam-sterilization was to prevent seed bank sprouting. We selected the 193 *A. thaliana* genotypes (Fig. 1a) based on their divergent genomic variation in regions associated with 129 genes linked with transport and utilization of N forms. These genes were listed using The *Arabidopsis* Information Resource (TAIR; https://www.arabidopsis.org/) and then used to filter *A. thaliana*'s single-nucleotide polymorphisms (SNPs) and produce a genomic distance matrix using PLINK 1.9 (Purcell *et al.*, 2007). This selection of accessions aimed to increase our chances of observing variation related to N metabolism.

We replicated each of the selected *A. thaliana* genotypes six times, assigning each replicate to one of six blocks. We irrigated the plants through subirrigation three times per week until the end of the experiment. Seventy days after sowing, we harvested three replicates per genotype plus 21 bare soil pots (i.e. same sampling date for 600 soil samples: 193 genotypes × 3 blocks + 21 bare soil samples). These soil samples were used to measure nitrifying (NEA) and denitrifying (DEA) enzyme activities, and to produce soil inocula for subsequent plant–soil feedback (PSF) analyses. NEA and DEA were measured across genotypes using soil harvested at the same time (70 d after sowing) to avoid confounding environmental factors that could affect microbial activities. The date of harvest was considered adequate based on previous studies that showed that *A. thaliana* is capable of rapidly recruiting soil microbial communities (Bukowski & Petermann, 2014). We harvested the three remaining replicates at flowering and used them exclusively to produce soil inocula for PSF analyses. Allowing three replicates to grow until flowering enabled us to obtain inocula per genotype that were representative of a broader growth period. We consider this approach the most conservative, since any relationship between soil enzyme activities and plant biomass production in home vs away soil tends to weaken with a growth period longer than 70 d.

For soil sampling, we cut plants at the rosette basis and collected all the contents of their pots (soil with roots). Such soil was mostly rhizospheric since the pots we used were small (0.08 l), and roots generally colonized most of the soil. For replicates harvested 70 d after sowing, we homogenized and divided the soil with roots into two equal parts, placing each part in a freezing bag. Half of the bags were directly frozen at −20°C for later use as inocula, and the other half were stored for a few days at 4°C until enzyme activities were measured. For replicates harvested at flowering, we homogenized the soil with roots and directly froze it at −20°C for later use as inocula.

Measurement of soil nitrifying and denitrifying enzyme activities

Nitrifying and denitrifying enzyme activities measures reflect the concentrations of soil nitrifying and denitrifying enzymes, with the assays occurring over a short period under optimal conditions for nitrification and denitrification, respectively (Niboyet *et al.*, 2011). Accordingly, they represent the potential ability of microbial communities to nitrify or denitrify, estimated through the rates of NO_2^- + NO_3^- and N_2O production, respectively, over time. Due to logistic issues, we could measure NEA and DEA respectively on 159 and 191 of the 193 initial genotypes, with 155 genotypes providing both NEA and DEA values.

Nitrifying enzyme activity was measured using the method described by Dassonville *et al.* (2011). Soil samples (3 g equivalent of dried soil) were placed in a flask with 21% O_2 atmosphere and supplemented with 30 ml of a water solution containing $(NH_4)_2$ SO_4 at 5 μg of N ml^{-1}. The flasks were incubated at 28°C and shaken at 144 rpm. The soil suspension was sampled and filtered every 2 h for 10 h. The amount of NO_2^- and NO_3^- produced during incubation was measured in samples using a SmartChem 200 photometer (AMS Alliance, Villeneuve-la-Garenne, France). We used the slope of the linear NO_3^--time regression to estimate NEA.

Denitrifying enzyme activity was measured according to Patra *et al.* (2006). Soil samples (10 g equivalent of dried soil) were placed in a 150-ml airtight plasma flask sealed with a rubber stopper. In each flask, the air was removed and replaced with a He/C_2H_2 mixture (90/10; v/v) to create anoxic conditions and inhibit N_2O-reductase activity. A nutrient solution containing glucose (0.5 mg of C g^{-1} of dried soil), glutamic acid (0.5 mg of C g^{-1} of dried soil), and potassium nitrate (50 μg of N g^{-1} of dried soil) was added to the soil to reach 100% of the water-holding capacity. The amount of N_2O in the headspace was measured 4, 5, 6, and 7 h after the start of the incubation at 28°C, using a gas chromatograph coupled with a microkatharometer detector (μGC-R990; SRA Instruments, Marcy l'Etoile, France). We used the slope of the linear regression

between the amount of N_2O produced per g soil and time to estimate DEA.

Reciprocal transplant experiment

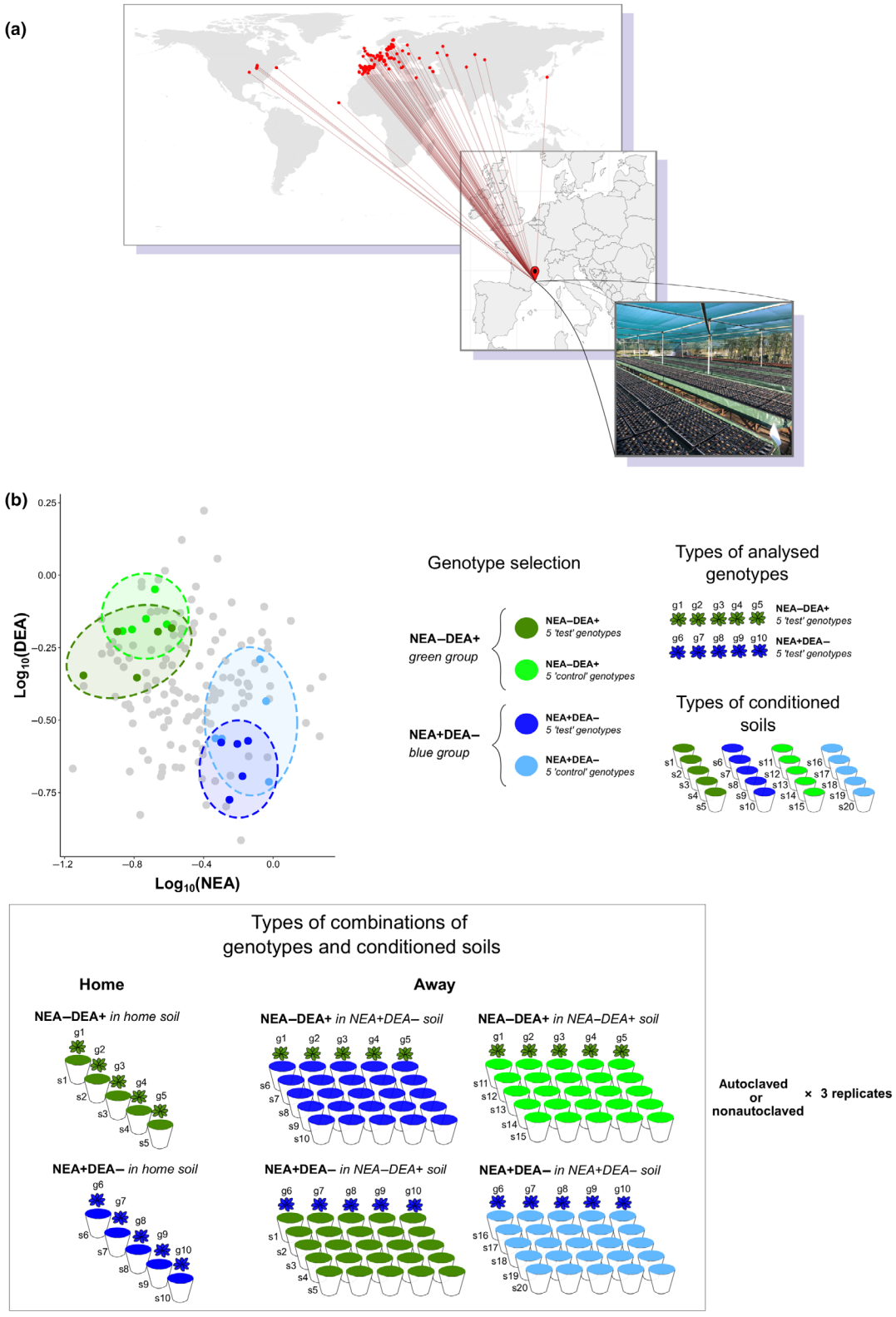
Among the 155 genotypes for which both NEA and DEA were measured, we selected 10 genotypes that were associated with high NEA and low DEA (called NEA^+DEA^-) and 10 genotypes that were associated with low NEA and high DEA (NEA^-DEA^+) for a reciprocal transplant experiment (NEADEA groups; Fig. 1b). This selection was based on contrasting values of NEA : DEA, since this ratio informs about the balance between nitrification and denitrification, which can impact plant nutrition by changing the NH_4^+-to-NO_3^- concentration in soil (Boudsocq *et al.*, 2012; Lata *et al.*, 2022). Accordingly, we selected these 10 NEA^+DEA^- and 10 NEA^-DEA^+ genotypes within the 25% tails of the distribution of NEA : DEA averaged per genotype (i.e. NEA : DEA > 1.57 and NEA : DEA < 0.41, respectively). Among these contrasting genotypes, we selected those for which we had enough soil from the previous experiment to be used as inocula. Our selection was independent of the geographic location of the genotypes and did not show geographic clustering (Supporting Information Fig. S1).

The reciprocal transplant experiment was conducted in 2022 at CEFE (Montpellier, France) to assess the transgenerational impacts of the plant influence on soil N cycling microbial activities through the analysis of plant biomass production in home vs away soil (intraspecific PSF). For that, we used a mixture of the soils that were conditioned by a given genotype during the common garden experiment to produce inocula (soils harvested 70 d after sowing and at flowering, that is 20 genotypes × 6 replicates), which were either autoclaved or not. This last step was necessary due to our focus on the transgenerational effects of plant-mediated N cycling microbial guilds, thus on the soil biotic component. We kept a low percentage of soil inoculation (which allowed to dilute any abiotic properties of the inoculum, to be described later) and either autoclaved (as a control) inocula or not to test for biotic PSF. Soil pooling to produce inocula was justified by our research questions, which focus on the influence of individual genotypes (accessions) on soil processes, rather than on the spatial variability of plant–soil interactions (Cahill *et al.*, 2017; Gundale *et al.*, 2017). Inocula were produced per genotype by defrosting, mixing, and subsequently dividing soils into two equal parts: one that was autoclaved (120°C for 20 min), and the other that was kept nonautoclaved. We then mixed inocula with steam-sterilized soil of the same composition used in the common garden (inoculation of 7% of the soil volume). The produced soil mixtures were used to fill a total of 660 pots (110 types of combinations of genotypes and conditioned soils × 2 autoclaving conditions × 3 replicates) of 0.08 l (Fig. 1b).

Because we had a limited amount of inoculum for each genotype and we wanted to achieve an inoculation of 7% (which allows to dilute any abiotic properties of the inoculum while ensuring a sufficient microbial effect; Brinkman *et al.*, 2010), we did not perform a fully factorial pairwise reciprocal transplant. Instead, we selected five genotypes of each NEADEA group (hereafter 'test' genotypes) to be reciprocally transplanted to pots inoculated either with their own soil (home soil) or with soil conditioned by 10 different genotypes (away soil): five belonging to the 'test' genotypes of the contrasting NEADEA group and five belonging to the same NEADEA group as the focal genotype (hereafter 'control' genotypes, Fig. 1b). 'Test' genotypes had their rosette biomass, hereafter aboveground biomass, measured in home vs away soil, while 'control' genotypes were only used to provide away soil within the same NEADEA group (Fig. 1b). Aboveground biomass was selected as a performance trait because of its effect on fitness in *A. thaliana* (Donohue, 2002; Postma & Ågren, 2022).

In April 2022, after incubating the pots for 2 d in the glasshouse (*c.* 20°C) to allow for the stabilization of soil microbial communities, we sowed 'test' genotypes and randomly distributed the pots in the same location where the common garden experiment was conducted the year before. We irrigated plants through subirrigation three times per week until the end of the experiment and also surveyed plant mortality. We recorded aboveground biomass for all samples at the end of the experiment (63 d after sowing) after drying the rosettes for 3 d at 60°C (Pérez-Harguindeguy *et al.*, 2016). As a measure of the mortality rate of each genotype in each type of conditioned soil, we calculated the ratio of dead plants at the end of the experiment to those alive halfway through (i.e. 1 month after sowing).

Fig. 1 Common garden and reciprocal transplant experiments using *Arabidopsis thaliana* genotypes. (a) The common garden experiment. One hundred and ninety-three *A. thaliana* accessions from a large geographic range were grown in the experimental field of Centre d'Ecologie Fonctionnelle et Evolutive (CEFE) in Montpellier, France. (b) The reciprocal transplant experiment. Among the 155 genotypes for which both nitrifying and denitrifying enzyme activities (NEA and DEA, respectively) were measured, we selected 10 genotypes that were associated with low NEA and high DEA (NEA^-DEA^+, green group) and 10 genotypes that were associated with high NEA and low DEA (NEA^+DEA^-, blue group). Within each NEADEA group, we selected five genotypes ('test' genotypes, dark green for NEA^-DEA^+ and dark blue for NEA^+DEA^-) that were reciprocally transplanted and had their aboveground biomass estimated in home vs away soil (here depicted through a rosette and identified with 'g' followed by a number); and other five ('control' genotypes, light green for NEA^-DEA^+ and light blue for NEA^+DEA^-) that were only used to provide away soil within the same NEADEA group. Types of conditioned soils are depicted through pots identified with 's' followed by the number of the genotype that conditioned them and through soil colour matching with genotype colour. We then combined the different 'test' genotypes with their home soil and with away soil from five 'test' genotypes of the contrasting NEADEA group and from five 'control' genotypes of the same NEADEA group. A total of 110 types of combinations of genotypes and conditioned soils were used in the experiment: 5 NEA^-DEA^+ genotypes in their home soil + 5 NEA^+DEA^- genotypes in their home soil + (5 NEA^-DEA^+ genotypes × 5 away soils from the contrasting NEADEA group) + (5 NEA^-DEA^+ genotypes × 5 away soils from the same NEADEA group) + (5 NEA^+DEA^- genotypes × 5 away soils from the contrasting NEADEA group) + (5 NEA^+DEA^- genotypes × 5 away soils from the same NEADEA group). The experiment had a total of 660 pots: 110 combinations of genotypes and conditioned soils × 2 autoclaving conditions × 3 replicates.



Statistical analyses

Because we analysed distinct *A. thaliana* wild accessions, population structure could explain part of the NEA, DEA, and NEA : DEA variation. To test such effect, while controlling for the block factor, we first ran linear mixed models for NEA, DEA, and NEA : DEA separately, assigning genetic group (a categorisation of *A. thaliana* accessions based on genetic distance; The 1001 Genomes Consortium, 2016; http://1001genomes.org/) and block as fixed factors, and genotype identity as random

factor. Response variables were $\log_{10}$-transformed to attain assumptions for parametric analyses. Because neither the effect of genetic group nor block was significant in any model, we excluded these factors from further analyses and used linear models to test the effect of genotype identity on each microbial enzyme activity separately.

After calculating least-square means per genotype for NEA, DEA, and NEA : DEA, we explored both the monogenic and polygenic architectures of these plant-mediated enzyme activities through Genome-Wide Association (GWA) studies. The procedures adopted for these analyses are fully described in Methods S1. We further used the 155 genotypes for which the NEA : DEA ratio was measured (the variable that presented the highest heritability according to the GWA analysis) to verify genetic differentiation between NEADEA groups (NEA^+DEA^- vs NEA^-DEA^+) and between each one of these groups and the remaining pool of genotypes (NEA^+DEA^- vs all other genotypes, except NEA^-DEA^+ genotypes; NEA^-DEA^+ vs all other genotypes, except NEA^+DEA^- genotypes). To this end, we calculated Weir and Cockerham's F_{ST} for each comparison according to procedures fully described in Methods S2. SNPs that had $F_{ST} \geq 0.5$ (i.e. the average threshold for obtaining the top 0.1% SNPs across F_{ST} distributions) were grouped in a Venn diagram to visualize common and singular SNPs among groups. To calculate the probability of obtaining by chance the same SNPs with $F_{ST} \geq 0.5$ as those obtained for NEA^+DEA^- vs NEA^-DEA^+, we conducted random F_{ST} comparisons (see Methods S2). Finally, using The *Arabidopsis* Information Resource (TAIR; https://www.arabidopsis.org/), we identified genes in the list of SNPs with $F_{ST} \geq 0.5$ for the comparison of NEA^+DEA^- vs NEA^-DEA^+. We then applied Gene Ontology (GO; Boyle *et al.*, 2004) to assess the functions of these genes, as well as of a subset of them that appeared in low frequency (≤ median) in the distribution of SNPs obtained through random F_{ST} comparisons.

To investigate if the conditioning of soil enzyme activities by *A. thaliana* was potentially adaptive, we explored the relationships between soil parameters at the geographic origin of the 193 studied accessions and NEA, DEA, and NEA : DEA measured in the common garden. For that, we used global pedologic layers obtained from ISRIC (Poggio *et al.*, 2021) to extract four soil parameters linked with N cycling: total N content; pH, which is one of the best predictors of microbial enzyme activities (Sinsabaugh *et al.*, 2008); bulk density, which is related to oxygen diffusion in soil (Asady & Smucker, 1989) thereby impacting nitrification and denitrification (predominantly aerobic and anaerobic processes, respectively); and organic carbon content, which affects soil fertility and directly impacts N cycling (Bothe *et al.*, 2006). Details about the extraction of these variables and linear model fitting are available in Methods S3.

During the reciprocal transplant experiment, one 'test' NEA^-DEA^+ genotype did not germinate in most of the pots (probably due to secondary seed dormancy) and had to be discarded from the analyses. We quantified, as a PSF measure, $\log_{10}$-response ratios comparing the 'test' genotypes' aboveground biomass in home vs away soil: $\log_{10}$ (aboveground biomass in home soil/aboveground biomass in away soil). To investigate if this measure was explained by differences in the genotypes' influence on enzyme activities during the common garden experiment, we used the least-square means of NEA, DEA, and NEA : DEA calculated earlier (see genomic analyses above) to compute ratios of home vs away enzyme activities for 'test' genotypes relative to other genotypes (those that conditioned away soil for 'test' genotypes). This calculation was conducted per enzyme activity and produced nine (because of the nongerminating genotype) and 10 ratios per NEA^+DEA^- and NEA^-DEA^+ 'test' genotype, respectively: the value of each enzyme activity in the focal genotype's home soil divided by the value of each enzyme activity in each one of the soils conditioned by five 'control' genotypes and each one of the soils conditioned by four or five 'test' genotypes. The effect of these ratios (soil conditioning in generation 1) on plant biomass production in generation 2 (PSF) was then tested through linear mixed models. In such models, $\log_{10}$-transformed ratios of each enzyme activity, autoclaving condition, and the interaction term were defined as fixed factors. The genotype for which PSF was analysed and the genotype that conditioned the soil were included as random factors. We also regressed the $\log_{10}$-transformed ratios of NEA, DEA, and NEA : DEA against PSF using the *sma* function of the SMATR R package (Warton *et al.*, 2012).

Finally, we performed a second PSF calculation that was based on mortality and corresponded to $\log_{10}$-response ratios comparing 'test' genotypes' mortality rate in away vs home soil. The reverse order of the factors in the ratios (away divided by home) was adopted since positive and negative PSFs had to, respectively, represent less and more mortality in home vs away soil. Because mortality rates per combination of genotype and conditioned soil contained null values, we added one to all rates before calculating PSF response ratios. To test for PSF differences between types of combinations of genotypes and conditioned soils, we used the Kruskal–Wallis nonparametric test. For *post hoc* comparisons, we applied the nonparametric Dunn test with Holm's correction, using the PMCMRPLUS R package (Pohlert, 2022).

All statistical analyses were performed using R 4.2.2 (R Core Team, 2022).

Results

Intraspecific variation in plant influence on N cycling enzyme activities

Nitrifying enzyme activity, DEA, and NEA : DEA ratio differed significantly among *A. thaliana* genotypes (Fig. 2, $P < 0.001$ for each variable). Many plant genotypes decreased NEA compared to bare soil, and a few increased this enzyme activity, leading to a *c.* 30-fold change in NEA (from 0.07 to 1.93 μg-N g^{-1} h^{-1}, Fig. 2a). For DEA, plant genotypes either decreased or increased this enzyme activity compared to bare soil, with a *c.* 15-fold change in DEA (from 0.12 to 1.71 μg-N g^{-1} h^{-1}, Fig. 2b). Many genotypes presented a NEA : DEA ratio that was higher than 1 and that generally followed the tendency observed for

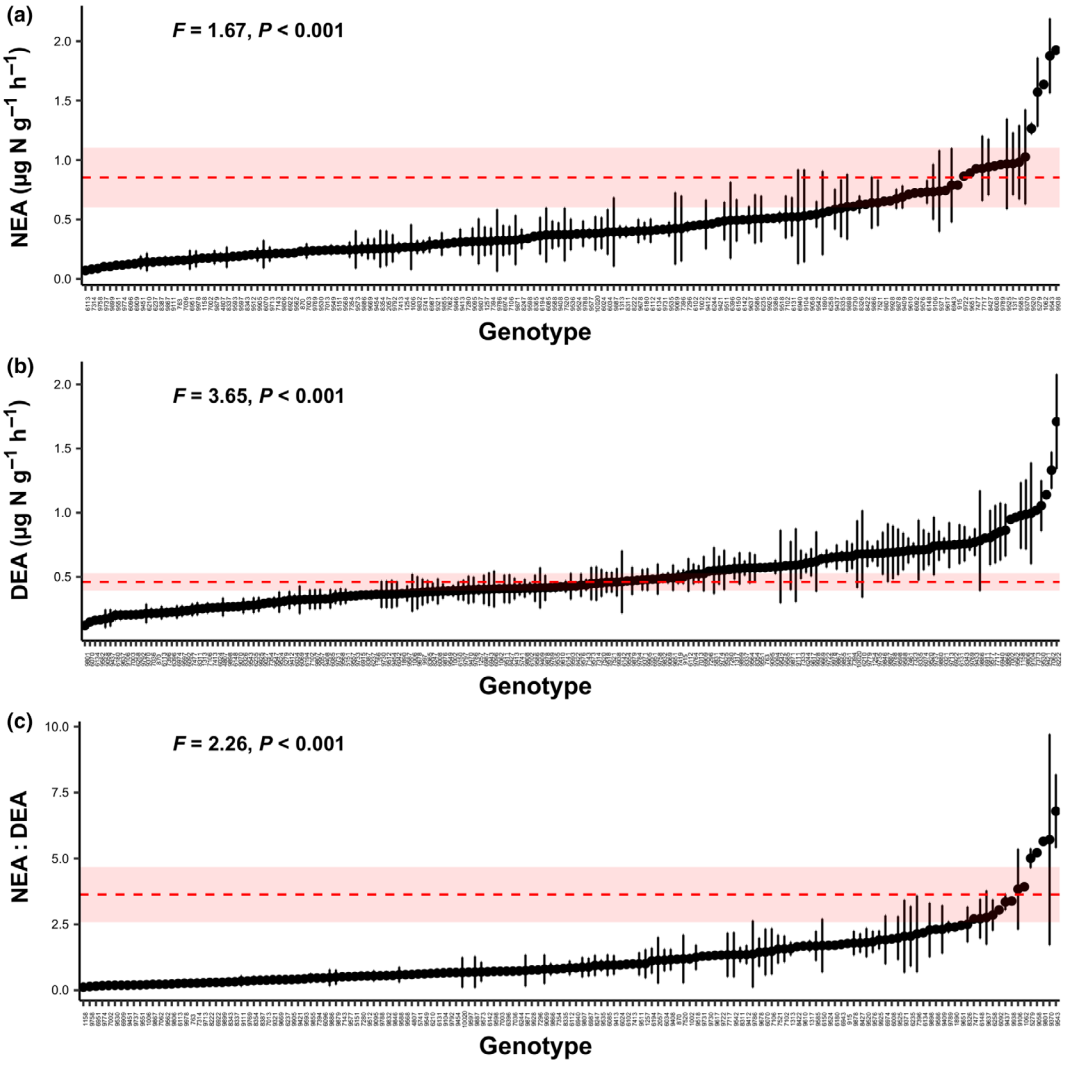


Fig. 2 Variation of nitrogen cycling enzyme activities in soil conditioned by different *Arabidopsis thaliana* genotypes grown in a common garden. Variation in nitrifying enzyme activity (NEA, a), denitrifying enzyme activity (DEA, b), and NEA-to-DEA ratio (NEA : DEA, c) is presented. Bars denote SE. The red dashed line and the red area denote, respectively, the mean value and the SE for each variable in bare soil. The Type II ANOVA test statistics for verifying the significance of the genotypic effect on each variable are provided.

NEA of lower values compared to bare soil, leading to a *c.* 60-fold change in NEA : DEA (from 0.12 to 6.80, Fig. 2c).

The genetic determinants of the influence of *A. thaliana* genotypes on N cycling enzyme activities

Monogenic Genome-Wide Association (GWA) studies did not reveal any significant SNP association with the plant influence on enzyme activities and their ratio. However, polygenic GWA revealed that NEA, DEA, and NEA : DEA were explained by many SNPs of weak effect (proportion of SNPs presenting a larger effect: pi ≤ 0.007%), with a heritability of 14%, 13%, and 16%, respectively, for each variable. F_{ST} analyses between contrasting groups, defined based on the NEA : DEA ratio (NEADEA groups), and between each one of these groups and all other genotypes revealed more SNPs with $F_{ST} \geq 0.5$ when NEADEA groups were compared with each other ($n = 1694$) than when they were compared with all other genotypes (Fig. 3; $NEA^{-}DEA^{+}$ vs other genotypes, $n = 420$; $NEA^{+}DEA^{-}$ vs other genotypes, $n = 224$).

We then conducted Gene Ontology (GO) with all the genes that differed between NEADEA groups ($n = 382$) and with those that appeared in low frequency (≤ median) in the distribution of SNPs obtained through random F_{ST} comparisons ($n = 209$). These analyses revealed enrichment for biological processes mostly associated with 'response to abiotic stimulus' (Fig. 3). Genes that presented very high F_{ST} ($F_{ST} \geq 0.8$) between NEADEA groups and a low frequency in the distribution of SNPs from random F_{ST} comparisons were linked with protein degradation, possibly related to abiotic stress (*AT1G73570*; Liu *et al.*, 2011; Su *et al.*, 2011), and with regulation of gene expression of the well-known *FLC* flowering locus (*AT5G40340*; Tables S1, S2). Other genes with $F_{ST} \geq 0.8$ had

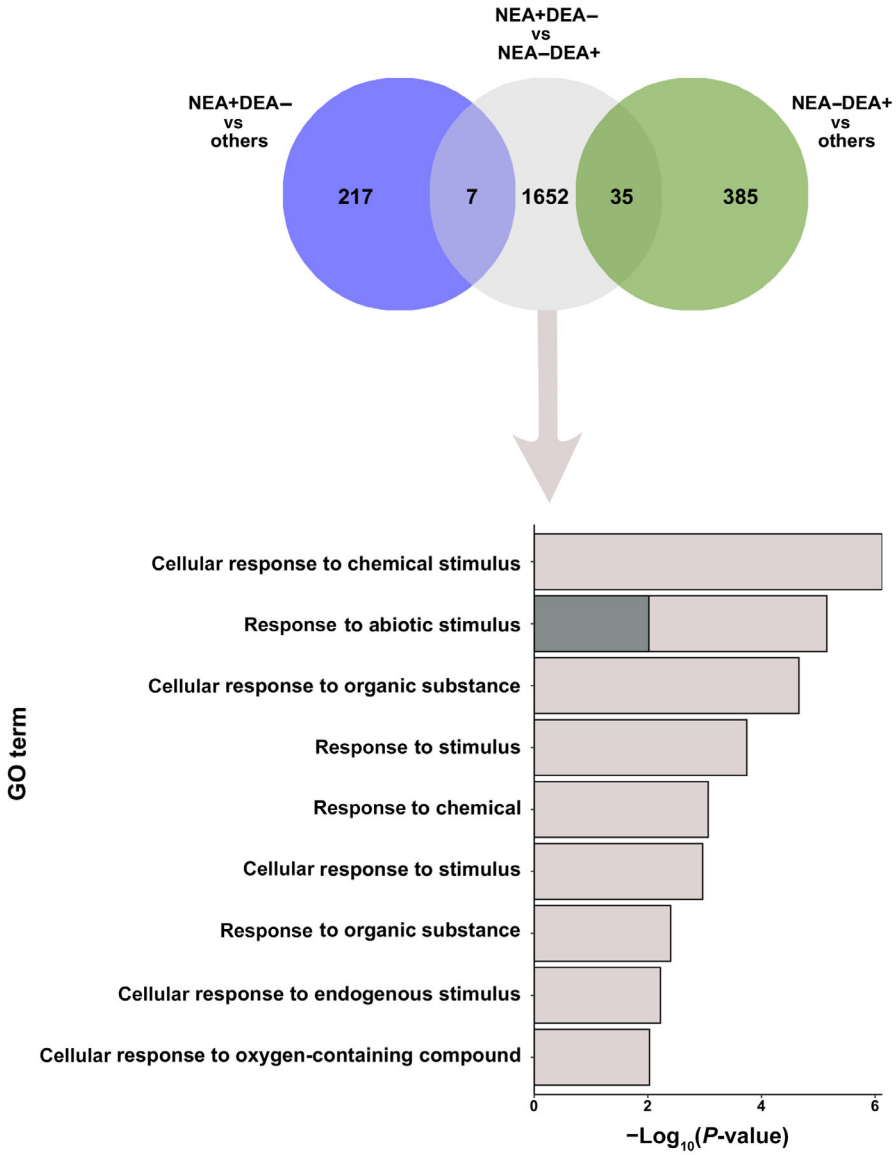


Fig. 3 Genomic variation associated with contrasting *Arabidopsis thaliana* genotypes in terms of their modulation of the ratio between soil nitrifying (NEA) and denitrifying (DEA) enzyme activities. Venn diagram for the association of single-nucleotide polymorphisms (SNPs) with $F_{ST} \geq 0.5$ when comparing genotypes that were associated with high NEA and low DEA ($NEA^{+}DEA^{-}$) vs all other genotypes (except those that were associated with low NEA and high DEA – $NEA^{-}DEA^{+}$; blue), $NEA^{+}DEA^{-}$ vs $NEA^{-}DEA^{+}$ genotypes (grey), and $NEA^{-}DEA^{+}$ genotypes vs all other genotypes (except $NEA^{+}DEA^{-}$ genotypes; green). We used Gene Ontology (GO) to identify the functions of the genes that were found within the 1652 most different SNPs between $NEA^{+}DEA^{-}$ and $NEA^{-}DEA^{+}$ groups, as well as within the subset of SNPs that appeared in low frequency (≤ median) in the distribution of SNPs obtained through random F_{ST} comparisons. Significant GO terms are provided for both analyses (in light and dark grey, respectively). Bar length indicates significance level ($-\log_{10}$(*P*-value)).

functions related to terpenoid biosynthetic process (*AT3G21500*) and pollen sperm cell differentiation (*AT4G11720*; Table S1). Similar functions were also found for some genes that had a low frequency in the distribution of SNPs from random F_{ST} comparisons (e.g. *AT1G26640* and *AT3G45130* are also involved in terpenoid biosynthetic process; Table S2). Furthermore, we verified which genes linked with transport and utilization of N forms were present in the distribution of SNPs with $F_{ST} \geq 0.5$ and found that those with the highest F_{ST} and a low frequency in the distribution of SNPs from random F_{ST} comparisons were *AT4G35270* and *AT1G32450*, that is genes linked with nitrate utilization and transport, respectively (Tables S2, S3).

The relationship between soil geographic parameters and plant-mediated N cycling enzyme activities

Soil total N content, pH, bulk density, and organic carbon at the geographic origin of *A. thaliana* accessions significantly correlated with DEA and NEA : DEA, and these relationships were particularly strong for the latter enzyme activity (Fig. 4a; Table S4). By contrast, NEA only presented a marginally significant relationship with soil bulk density ($P = 0.074$, Table S4). While soil total N content and organic carbon were positively correlated with NEA : DEA, soil pH and bulk density were negatively correlated with this ratio (Fig. 4a). Furthermore, soil pH, bulk density, and organic carbon differed, either significantly or marginally significantly ($P < 0.05$, $P = 0.054$, $P = 0.090$, respectively), among groups of genotypes contrasting in terms of their modulation of NEA : DEA ($NEA^{+}DEA^{-}$, $NEA^{-}DEA^{+}$, and other genotypes; Fig. 4b; Table S5). Soil total N content, in turn, did not differ among these groups ($P = 0.239$; Fig. 4b; Table S5). The soil at the geographic origin of $NEA^{-}DEA^{+}$ genotypes had higher pH, higher bulk density, and lower organic carbon than the soil from where $NEA^{+}DEA^{-}$ genotypes came from (Fig. 4b). Soil pH was even higher for $NEA^{-}DEA^{+}$ genotypes than for all other analysed genotypes (Fig. 4b).

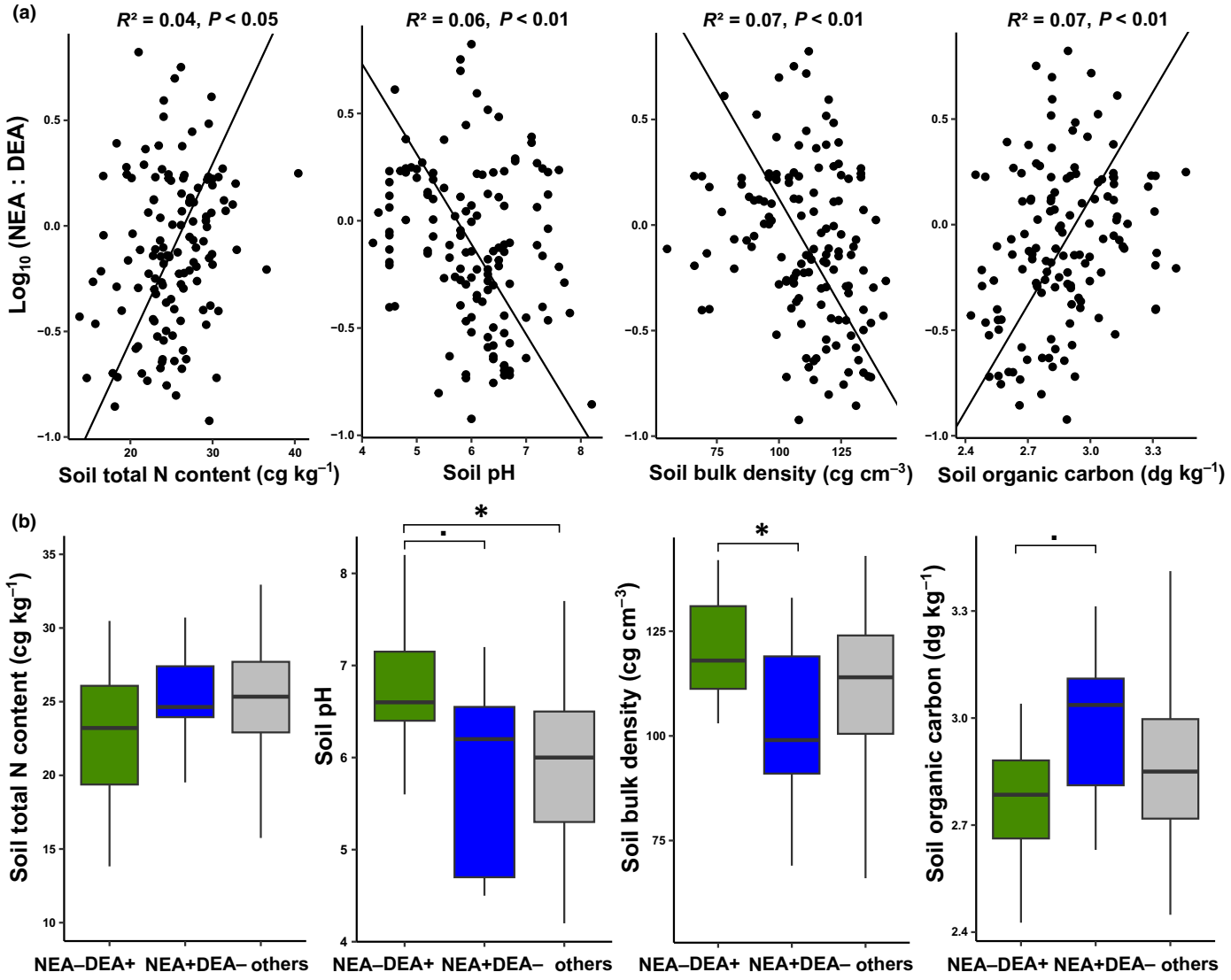


Fig. 4 Relationships between soil geographic parameters and the ratio of plant-mediated soil nitrifying and denitrifying enzyme activities (NEA : DEA) across *Arabidopsis thaliana* genotypes. (a) Correlations between soil geographic parameters and the NEA : DEA ratio modulated by *A. thaliana* accessions (n = 193). Note square-root scale for soil total nitrogen content and $\log_{10}$ scale for soil organic carbon. (b) Boxplots of soil geographic parameters across groups of *A. thaliana* accessions contrasting in terms of their modulation of the NEA : DEA ratio: accessions associated with low NEA and high DEA (green, $NEA^{-}DEA^{+}$, n = 10), accessions associated with high NEA and low DEA (blue, $NEA^{+}DEA^{-}$, n = 10), and all other analysed accessions (grey, n = 135). The coloured area within the boxplots corresponds to the interquartile range (IQR), and the dark horizontal line inside corresponds to the median. Vertical lines represent data variability outside the IQR (1.5 times the IQR from the quartiles). Asterisks denote significance based on Tukey HSD tests. *, $P < 0.05$. Dots denote marginal significance: P = 0.058 (soil pH) and P = 0.073 (soil organic carbon). Note square-root scale for soil total nitrogen content and $\log_{10}$ scale for soil organic carbon.

Transgenerational effects of plant-mediated N cycling enzyme activities

N cycling enzyme activities in soil conditioned by different genotypes of *A. thaliana* impacted the biomass production of these genotypes in a subsequent generation (i.e. plant performance). Specifically, NEA and NEA : DEA in home vs away soil (i.e. soil conditioning in generation 1), and their interaction with autoclaving condition, had significant effects ($P < 0.05$, Table S6) on genotypes' aboveground biomass in home vs away soil (i.e. plant performance in generation 2, plant–soil feedback, PSF). By contrast, DEA in home vs away soil and its interaction with autoclaving condition had only marginally significant effects on plant aboveground biomass in home vs away soil (P = 0.058 and P = 0.083, respectively; Table S6). In line with these results, NEA, DEA, and NEA : DEA in home vs away soil significantly correlated with genotypes' aboveground biomass in home vs away soil only when this was not autoclaved (Fig. 5). The strength of the correlation was higher for NEA and NEA : DEA compared to DEA (Fig. 5). Accordingly, plant biomass production in generation 2 was influenced by NEA, NEA : DEA and, to a lesser extent, DEA only when the biotic composition selected during

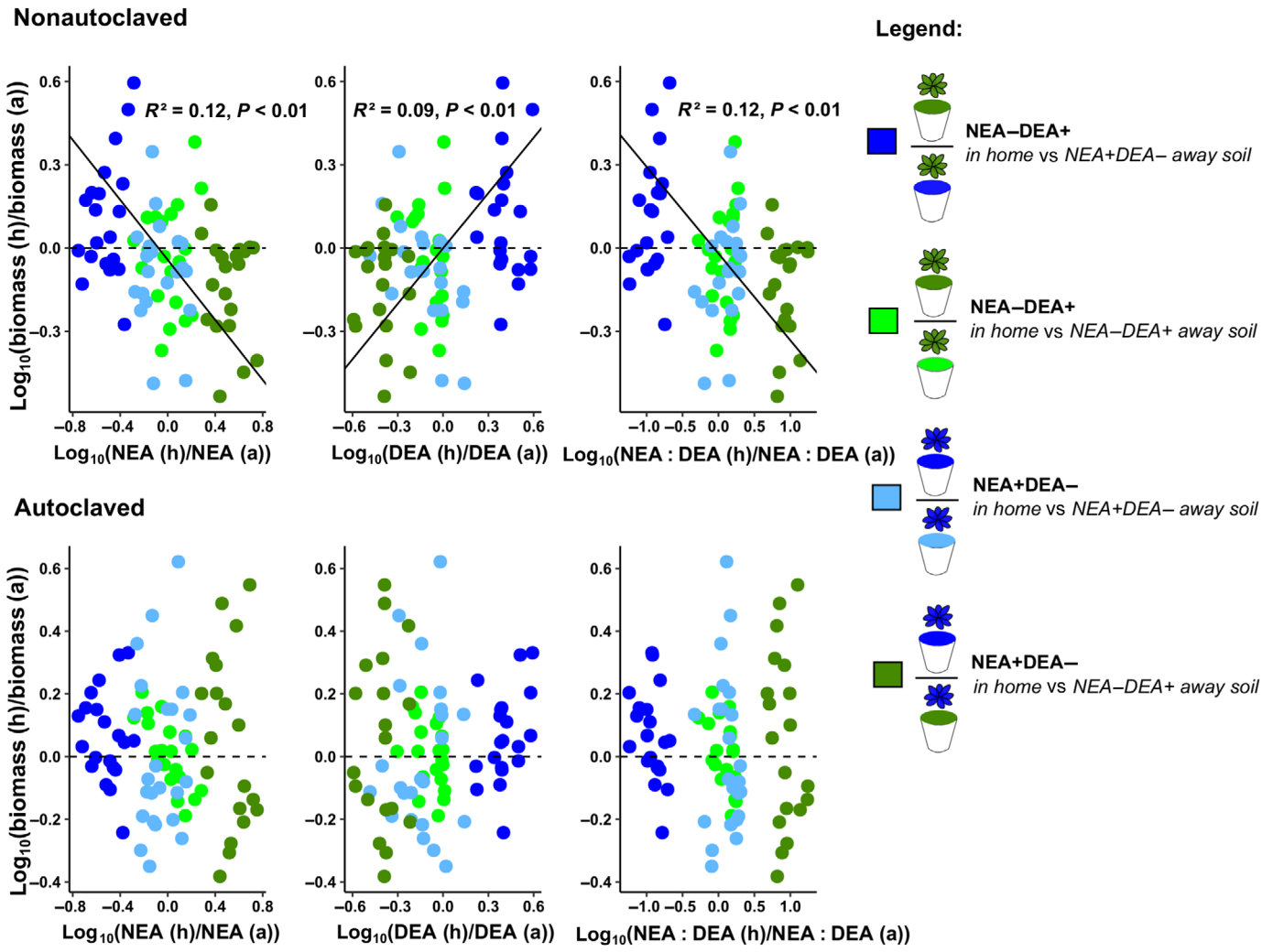


Fig. 5 Relationships between plant–soil feedback (PSF) and nitrogen cycling enzyme activities in home vs away soil in *Arabidopsis thaliana*. Ratios of home vs away nitrifying and denitrifying enzyme activities (NEA and DEA, respectively), and their ratio (NEA : DEA) were regressed against PSF. Plant–soil feedback was measured as $\log_{10}$-response ratios comparing 'test' genotypes' aboveground biomass (biomass) in home (h) vs away (a) nonautoclaved (upper panel) and autoclaved (lower panel) soils. It was assessed in the reciprocal transplant experiment (generation 2), while enzyme activities were measured in the common garden experiment (generation 1). Plant–soil feedback of each 'test' group of genotypes, contrasting in terms of the modulation of NEA : DEA (NEADEA group), in each type of conditioned soil is depicted with different colours: dark blue, 'test' genotypes associated with low NEA and high DEA (NEA^-DEA^+) in soil conditioned by 'test' genotypes associated with high NEA and low DEA (NEA^+DEA^-); light green, 'test' NEA^-DEA^+ genotypes in soil conditioned by 'control' NEA^-DEA^+ genotypes; light blue, 'test' NEA^+DEA^- genotypes in soil conditioned by 'control' NEA^+DEA^- genotypes; dark green, 'test' NEA^+DEA^- genotypes in soil conditioned by 'test' NEA^-DEA^+ genotypes. Lines were fitted with SMA regressions. R^2 denotes the coefficient of determination. Enzyme activities and PSF were not significantly correlated in autoclaved soils.

previous plant growth was preserved. Additionally, in nonautoclaved away soil conditioned by the contrasting NEADEA group, NEA^-DEA^+ plants presented more positive PSFs than NEA^+DEA^- plants (Fig. 5). This relative home disadvantage of NEA^+DEA^- genotypes was also evidenced through the mortality assessment in away vs home soil ($\chi^2 = 20.1$, $P < 0.01$). The relative mortality of NEA^+DEA^- plants in nonautoclaved away soil conditioned by NEA^-DEA^+ genotypes vs nonautoclaved home soil was significantly lower than the relative mortality of NEA^-DEA^+ plants in nonautoclaved away soil conditioned by NEA^+DEA^- or other NEA^-DEA^+ genotypes vs nonautoclaved home soil ($P < 0.01$ and $P < 0.05$, respectively; Fig. S2). The home disadvantage of NEA^+DEA^- plants significantly decreased with soil autoclaving ($P < 0.05$, Fig. S2).

Discussion

Intraspecific variation in the influence of plants on specific soil N cycling processes and its evolutionary consequences have been mostly studied in the context of symbiotic interactions. For instance, a number of studies have investigated the performance of different plant genotypes in response to soil inoculated with single or multiple strains of symbionts fostering plant nutrition (e.g. Heath, 2010; Johnson *et al.*, 2010; Rekret & Maherali, 2019;

Epstein *et al.*, 2023). Here, we demonstrated that genotype-based modulation of soil N cycling may go beyond symbiotic interactions and entail rapid evolutionary consequences. We found evidence that globally distributed *A. thaliana* genotypes strongly differed in the way they influenced soil nitrifying (NEA) and denitrifying (DEA) enzyme activities, and their ratio (NEA : DEA). This intraspecific variation was partly genetically based and linked with soil geographic parameters. Moreover, we found that N cycling enzyme activities (mostly NEA and the NEA : DEA ratio) correlated with the aboveground biomass of next-generation plants in home vs away nonautoclaved soil, suggesting that the biotic conditioning of soil by *A. thaliana* genotypes can have transgenerational impacts on plant performance.

Nitrifying and denitrifying enzyme activities varied significantly across soils conditioned by different *A. thaliana* genotypes. For NEA, this variation was comparable to variation recorded for different herbaceous species growing alone (Cantarel *et al.*, 2015), in monocultures or in different mixtures (Le Roux *et al.*, 2013). Though there is growing evidence that *A. thaliana* genotypes differentially modify their rhizosphere microbiome (Micallef *et al.*, 2009; Lundberg *et al.*, 2012; Bergelson *et al.*, 2019; Huang *et al.*, 2019; Kudjordjie *et al.*, 2021), little is known about how they modify soil microbial activities related to nutrient cycling. For instance, while N mineralization by bacteria of the *Pseudomonas* genus has been shown to affect *A. thaliana* performance (Weidner *et al.*, 2015), intraspecific variation in the recruitment of N mineralizing microbial communities has not been investigated. Regarding other N cycling processes, genotype-dependent recruitment of nitrifying and denitrifying microbial communities has been demonstrated in *A. thaliana*, but only for one wild accession compared with a mutant (Lu *et al.*, 2018). The large variation in nitrification and denitrification modulation that we found here indicates that there is genetic variation on which natural selection can act.

Since genetic inheritance can facilitate the legacy of a conditioned soil through generations (Odling-Smee *et al.*, 2013), we investigated the genetic determinism of NEA, DEA, and NEA : DEA in *A. thaliana*. In accordance with previous studies investigating the root microbiome (Peiffer *et al.*, 2013; Bergelson *et al.*, 2019; Huang *et al.*, 2019), we found a polygenic architecture for these variables characterized by many genes of weak effect and low heritability. Accordingly, no single genes sufficiently explain the recruitment of rhizospheric microorganisms by *A. thaliana*, including its capacity to modulate NEA and DEA. It is possible that metabolic networks underpinned by multiple genes are the rule in plant–microbe interactions. This is because plants produce a variety of root exudates that specifically drive microbial communities in the rhizosphere (Zhalnina *et al.*, 2018; J-M. Chen *et al.*, 2022) and that might be the product of biosynthetic networks controlled by clusters of genes (Huang *et al.*, 2019). Identifying the genomic regions that were most strongly associated with the here-identified genotypes that differently modulated the balance between NEA and DEA may shed light on key elements of these putative metabolic networks. For instance, we detected that the genes that highly differed between NEADEA groups in a nonrandom fashion were involved in protein degradation, possibly related to abiotic stress (*AT1G73570*; Liu *et al.*, 2011; Su *et al.*, 2011), and regulation of gene expression (*AT5G40340*). Interestingly, *AT5G40340* is linked with the regulation of *FLC* expression, a major repressor of flowering (Michaels & Amasino, 1999). Moreover, we found two candidate genes related to NO_3^- utilization and transport that significantly differed between NEADEA groups (*AT4G35270* and *AT1G32450*). In accordance with these results, plant-mediated nitrifying microorganisms have been previously shown to influence soil NO_3^- availability with impacts on flowering onset in *A. thaliana* (Lu *et al.*, 2018). Altogether our findings are in line with the idea of a complex chemical communication between plants and soil microbes impacting plant responses to stress and, in particular, to nutrient limitation (Rolfe *et al.*, 2019; Rizaludin *et al.*, 2021).

Supporting a putative adaptive role for *A. thaliana*'s ability to modulate N cycling enzyme activities, we found that the NEA : DEA ratio significantly correlated with soil geographic parameters and that the soil at the origin of contrasting accessions in terms of NEA : DEA (NEADEA groups) had different values of pH, bulk density, and, to a lesser extent, organic carbon. NEA^-DEA^+ genotypes were associated with higher soil pH and bulk density and lower soil organic carbon than NEA^+DEA^- genotypes. Surprisingly, soil total N content did not significantly differ between NEADEA groups, which suggests that the soil content of specific inorganic N forms, such as NH_4^+ and NO_3^-, might have been more informative in this case (but was not available in the used dataset). Increased nitrification and denitrification rates have been reported in neutral to alkaline soils (Šimek & Cooper, 2002; Hayatsu *et al.*, 2008; Bardon *et al.*, 2018). Therefore, a plant strategy of decreasing the nitrification rate, and hence preventing N loss through NO_3^- leaching (which is negatively charged and hence poorly retained in the soil; Subbarao *et al.*, 2015) and/or denitrification, might be evolutionarily favoured in alkaline soils with low fertility caused by low soil organic carbon content and low oxygen diffusion (resulting from high bulk density; Asady & Smucker, 1989), such as the one that was associated with NEA^-DEA^+ genotypes. Conversely, stimulating nitrification might be evolutionarily favoured in acidic soils with high organic carbon content and oxygen diffusion, such as the one that we found to be associated with NEA^+DEA^- genotypes. However, the capacity of modulation of N cycling enzyme activities in plants adapted to alkaline stress has been much less studied than in plants adapted to acidic conditions, and further research on the environmental variables linked with N cycling microbial modulation by plants is necessary (Wang *et al.*, 2023).

Pursuing the investigation of the adaptive role of plant–soil interactions, we tested if N cycling modulation by *A. thaliana* had transgenerational effects on its performance. We found that plant biomass production significantly correlated with soil NEA, NEA : DEA, and, to a lesser extent, DEA (conditioned by a previous generation) only when the biotic composition of the soil was kept unaltered (i.e. nonautoclaved). In particular, N cycling enzyme activities significantly correlated with the biomass production of next-generation plants (plant–soil feedback, PSF) due to a difference in aboveground biomass of NEA^+DEA^- and

$NEA^{-}DEA^{+}$ genotypes in home vs away soil. The former presented negative biotic PSF (low aboveground biomass in nonautoclaved home vs away soil) while the latter presented positive biotic PSF (high aboveground biomass in nonautoclaved home vs away soil). A similar result was obtained for PSF calculated through mortality, which suggests that the biotic conditioning of soil N cycling by *A. thaliana* entails rapid evolutionary consequences through niche construction. However, the transgenerational response was stronger for NEA and the NEA : DEA ratio than DEA, a pattern that may be explained by both the higher heritability recorded for the former enzyme activities and the intrinsic dependency of denitrification on the NO_3^- supply provided by nitrification (Bothe *et al.*, 2006; Chapin *et al.*, 2011).

Previous studies have already demonstrated that soil modified by *A. thaliana* genotypes can have transgenerational impacts (Bukowski & Petermann, 2014; Lu *et al.*, 2018; Kalachova *et al.*, 2023), but the role of specific N cycling processes in intraspecific PSFs remains less studied (but see Lu *et al.*, 2018). Two potential explanations for the transgenerational pattern that we observed can be evoked. First, it is possible that inhibition and stimulation of nitrifiers by $NEA^{-}DEA^{+}$ and $NEA^{+}DEA^{-}$ plants, respectively, in generation 1 favoured a new soil NH_4^+-to-NO_3^- balance in generation 2, which affected the biomass production of NEADEA plants. This is particularly probable if these plants have distinct affinities for NH_4^+ and NO_3^-, a characteristic that has been previously observed in plants with the capacity to release biological nitrification inhibitors (BNIs; Lata *et al.*, 1999; Boudsocq *et al.*, 2012). In particular, if $NEA^{-}DEA^{+}$ plants present high affinity for NH_4^+ (the N form favoured under nitrification inhibition; Boudsocq *et al.*, 2012; Subbarao *et al.*, 2015) while $NEA^{+}DEA^{-}$ plants have affinity for both N forms (which are both expected to be available with increasing nitrification; Boudsocq *et al.*, 2012), both $NEA^{-}DEA^{+}$ and $NEA^{+}DEA^{-}$ plants would benefit from a potentially high NH_4^+-to-NO_3^- balance favoured by $NEA^{-}DEA^{+}$ conditioning. By contrast, nitrification stimulation and the potentially consequent low NH_4^+-to-NO_3^- ratio might be relatively disadvantageous not only for $NEA^{-}DEA^{+}$ but also for $NEA^{+}DEA^{-}$ plants grown in poor soils, such as the one used in our experiments, due to the leaching tendency of NO_3^- (Subbarao *et al.*, 2015). Though we do not know whether *A. thaliana* is capable of releasing BNIs, genes related to terpenoid biosynthetic process either presented very high F_{ST} or nonrandom variation in our genetic comparison of $NEA^{+}DEA^{-}$ vs $NEA^{-}DEA^{+}$ genotypes (*AT3G21500*, *AT1G26640*, and *AT3G45130*). Terpenoid compounds have been previously shown to be involved in the modulation of the *A. thaliana* root microbiome (Huang *et al.*, 2019), as well as to present allelopathic effect and, for some of them, the capacity to inhibit nitrification (Bremner & McCarty, 1993; Langenheim, 1994; Adamczyk *et al.*, 2013; Coskun *et al.*, 2017). Moreover, we also identified genes related to NO_3^- transport and utilization (as previously mentioned) that can be promisingly investigated in future studies about the connexions between plant NH_4^+ vs NO_3^- affinity and biological nitrification inhibition. Second, the observed transgenerational soil effects could be indirect through a concurrent plant recruitment of pathogens and/or mutualists. These microorganisms have not been evaluated in our study but have been repeatedly linked with negative and positive PSF, respectively (Semchenko *et al.*, 2022). Some studies have notably shown an association of plant growth-promoting bacteria (PGPRs) with soil denitrification rate (Florio *et al.*, 2017, 2019), indicating that more complex biotic interactions related to N cycling are possible.

By demonstrating that *A. thaliana* genotypes are capable of modulating N cycling microbial activities and of impacting their own performance and that of other genotypes, we show that the conditions for plant niche construction through soil legacy are fulfilled. This opens compelling perspectives for the study of evolution over short time scales. Furthermore, in agroecology, mixtures combining genotypes that promote positive PSF with other that do not or, more specifically, that inhibit (de)nitrification with others that do not may allow coexistence and favour yield in crop systems (Barot *et al.*, 2017; Mariotte *et al.*, 2018; Jing *et al.*, 2022). However, because correlation does not mean causation, further studies are necessary to help clarify the ecological and evolutionary determinants of plant control over N cycling processes. For instance, evidence that soil legacy lasts for multiple generations is important (Odling-Smee *et al.*, 2013). Moreover, the precise physiological mechanisms and the direct environmental drivers involved in genotype-based NEA, DEA, and NEA : DEA modulation remain to be elucidated. Finally, because NEA and DEA are measures taken in optimal conditions, they might not reflect what is specifically occurring in natural conditions, in which they are known to fluctuate with abiotic factors (Attard *et al.*, 2011). Therefore, the investigation of soil microbe-mediated N fluxes (e.g. through ^{15}N pool dilution; Murphy *et al.*, 2003) coupled with the quantification of the abundance of soil N cycling microbial groups are steps that will allow for a more precise characterization of the microbial communities in interaction with the contrasting *A. thaliana* genotypes described here. Overall, the potentially ubiquitous capacity of plants to construct their niche in order to satisfy specific nutrient needs reveals their active role in evolution and must change the way we perceive, study, and manage plants.

Acknowledgements

This work was supported by Agence Nationale de la Recherche (ANR; www.anr.fr) and Deutsche Forschungsgemeinschaft (DFG; www.dfg.de) funding as part of the AraBreed project (Grants ANR-17-CE02-0018-01 and SCHE 1899/2-1). CV was supported by the European Research Council (ERC) Starting Grant Project 'Ecophysiological and biophysical constraints on domestication in crop plants' (Grant ERC-StG-2014-639706-CONSTRAINTS). FV was supported by the European Research Council (ERC) Starting Grant Project 'PHENOVIGOUR' (Grant ERC-StG-2020-949843). We are grateful to the 'Terrain d'expériences' platform at CEFE, namely to Thierry Mathieu, David Degueldre, Pauline Durbin, Fabien Lopez, Pierrick Aury, and Jean-Marc Donnay, for supporting the conception and execution of the experiment. We thank Lou Sales-Mabily, Pierre Moulin, Robin Latapie, Annick Lucas, and Rahma Kazi-Tani for

help during the experiment, as well as David Lun for help during (de)nitrification enzyme activity assays, part of an internship at LEM, and Jonathan Gervaix for his technical support. NEA and DEA were quantified at the 'Microbial Activities in the Environment' AME platform of LEM (Villeurbanne, France). We are grateful to Amélie Cantarel for providing valuable feedback on the manuscript. We are also grateful to Moises Exposito-Alonso for providing seeds for the experiment and Damien de La Faye for help with figure conception and drawing design.

Competing interests

None declared.

Author contributions

MSP, CV, DV, XLR and FV conceived the study in collaboration with JFS, FM and MV. MSP, XLR and FV coordinated the experiments. MSP carried out the common garden experiment, and MSP and MV carried out the reciprocal transplant experiment. AT performed the measurement of soil nitrifying and denitrifying enzyme activities. MSP analysed the data, wrote the manuscript, and produced figures and tables in collaboration with FV, XLR, CV and DV. All authors read and improved the manuscript. XLR and FV contributed equally to this work.

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Data availability

The data that support the findings of this study are openly available in the data.InDoRES repository at doi: 10.48579/PRO/FLP2AD.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Geographic distribution of the *Arabidopsis thaliana* accessions analysed for plant–soil feedback.

Fig. S2 Boxplots of plant–soil feedback based on mortality for different combinations of *Arabidopsis thaliana* genotypes and conditioned soils.

Methods S1 Genome-Wide Association studies.

Methods S2 F_{ST} analyses.

Methods S3 Environmental analyses.

Table S1 *Arabidopsis thaliana*'s single-nucleotide polymorphisms and genes presenting very high F_{ST} ($F_{ST} \geq 0.8$).

Table S2 *Arabidopsis thaliana*'s single-nucleotide polymorphisms (SNPs) and genes presenting $F_{ST} \geq 0.5$ and a low frequency ($\leq$ median) in the distribution of SNPs obtained through random F_{ST} comparisons.

Table S3 *Arabidopsis thaliana*'s single-nucleotide polymorphisms (SNPs) and genes linked with transport and utilization of nitrogen forms that were in the list of SNPs with $F_{ST} \geq 0.5$.

Table S4 The results of Type II ANOVA tests of the effects of soil geographic parameters on soil enzyme activities modulated by 193 *Arabidopsis thaliana* accessions.

Table S5 The results of Type II ANOVA tests comparing soil geographic parameters across groups of *Arabidopsis thaliana* accessions.

Table S6 The results of Type III ANOVA tests of the effects of plant-mediated microbial enzyme activities, autoclaving condition, and their interaction on plant–soil feedback in *Arabidopsis thaliana*.

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