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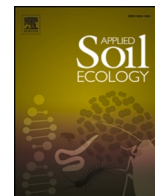
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## Research paper

# Impact of two acquisitive plants on N cycle on different soils: The invasive *Fallopia japonica* does it and so does the native *Dactylis glomerata*!

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## ABSTRACT

Invasive plants may alter ecological and ecosystem processes, including the N-cycle. The *Fallopia* species complex is a well-studied invasive species whose N-resource acquisition traits define it as an acquisitive species. However, the study of the impacts of invasive plants on the N-cycle never considers the N-acquisition strategy as a reference for choosing another suitable plant control. The purpose of this study is to assess the impacts of an invasive species (*Fallopia japonica* — *FJ*) on the N-cycle and to compare with those caused by a native acquisitive species (*Dactylis glomerata* — *DG*), all compared to unplanted soils. A four-months mesocosm experiment was conducted by growing *FJ* and *DG* on nine different soils and measuring their impacts on N-cycle microbial activities (free-living nitrogen fixation — FLNF, denitrification — DEA, nitrification — NEA), on N-mineral forms and on functional N-cycle gene abundance (*nifH*, *AOA*, *AOB*, *nirS*, *nirK*) as well as the total bacterial community gene (*rRNA 16S*). The nine soils differ in microbial enzymatic activities, N-mineral form concentrations, physico-chemical factors, texture, and gene abundances. Plant effects on FLNF, NEA and DEA are only soil dependent and no effect of invasive status was found. In addition, the native plant *DG* generally affected microbial parameters over a wider range of soils than the invasive plant. Stronger impacts of the native *DG* on microbial gene abundances were found compared to the invasive *FJ*. A stronger effect of the invasive plant was found for the soil  $\text{NO}_3^-$  concentration, with a significant decrease under the *FJ* than under the *DG*. Under both *FJ* and *DG*,  $\text{NH}_4^+$  concentrations were not significantly affected. In conclusion, the invasive status in the ecosystem of the two plants studied cannot be explained through their impacts on microbial enzymatic activities and gene abundances of the soil N-cycle and the soil N mineral pools.

## 1. Introduction

It is widely accepted that invasive plant species can alter ecological and ecosystem processes (Ehrenfeld, 2010). Some studies report changes in soil functioning and soil biogeochemical cycles caused by invasive species (Ehrenfeld, 2003; Torres et al., 2021). These changes may result from fluctuations in physico-chemical parameters or in soil nutrient pools, reflecting a shift in the quantity and distribution of nutrients in the ecosystem (Osunkoya and Perrett, 2011; Ahmad et al., 2019; Araya et al., 2022). Many studies have also demonstrated changes in abundance, diversity and composition of soil microbial communities (Elgersma and Ehrenfeld, 2011; Si et al., 2013; Torres et al., 2021).

These effects can lead to changes in soil microbial processes. Consequently, the effects of invasive plants on the carbon (C) and nitrogen (N) biogeochemical cycles are particularly significant (Liao et al., 2008). Some ecologists consider that invasive species “are defined by their negative impact” on ecosystem processes (Russell and Blackburn, 2017). Nevertheless, the semantic field of the concept of biological invasion, the idea of native range and the interplay between the context of invasions and the sources of variability, particularly environmental, are currently the focus of debate (Hulme et al., 2013; Warren et al., 2017; Courchamp et al., 2020; Pereyra, 2020; Pereyra and Guiaşu, 2020).

All plants can strongly influence N-cycle and N microbial diversity in soil, mainly in the rhizosphere, by modifying soil properties including N

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**Table 1**

Soil characteristics (microbial enzymatic activities, N-mineral form concentrations, physico-chemical factors, texture, and gene abundances) before the experiment (i.e., initial soil parameters) on unplanted soils. Soil names are explained in Table S1.

Parameters	Units	Soils									
		AG	CF	G	LCSA	Lo	S	STA	STM	V	
Free-Living Nitrogen Fixation (FLNF)	ng N-NH <sub>3</sub> /g dry soil/h	43.28	84.57	38.48	16.16	51.46	33.82	0.87	34.71	0.40	
Nitrification (NEA)	μg N-NO <sup>3-</sup> + NO <sub>3</sub> <sup>-</sup> /g dry soil/h	1.61	1.26	1.41	1.16	2.08	0.84	0.12	1.74	0.07	
Denitrification (DEA)	μg N-N <sub>2</sub> O/g dry soil/h	3.57	1.87	3.06	0.8	2.71	1.5	1.49	3.81	0.78	
Substrate-induced respiration (SIR)	μg C-CO <sub>2</sub> /g dry soil/h	17.83	25.66	28.51	13.06	17.63	15.85	19.84	25.75	12.27	
Ammonium (NH <sub>4</sub> <sup>+</sup> )	μg N-NH <sub>4</sub> <sup>+</sup> /g dry soil	0.296	0.288	0.244	0.172	0.124	0.192	0.356	0.208	2.268	
Nitrate (NO <sub>3</sub> <sup>-</sup> )	μg N-NO <sub>3</sub> <sup>-</sup> /g dry soil	21.1	19.6	11.6	9.2	21.5	26.5	30.6	4.5	8.2	
pH		8.16	7.35	6.89	6.76	8.17	7.24	4.9	8.01	4.38	
Moisture	% for 100 g of dry soil	28.26	24.07	18.02	17.76	19.56	11.67	26.83	16.49	15.47	
Soil Texture	Jamagne diagram (1967)	Sandy-clay loam	Sandy loam	Sandy-clay loam	Sandy-clay loam	Loamy sand	Sandy-clay	Sandy-clay	Sandy-clay loam	Medium sandy loam	
Clays	g/kg	178	143	235	176	114	173	160	245	108	
Silts		277	324	310	375	209	183	259	304	707	
Sands		545	533	455	450	676	644	581	451	185	
Organic matter (OM)		62.25	107.01	69.56	24.52	59.22	21.53	53.69	59.93	66.18	
Total Carbon (C)		36.19	62.22	40.44	14.26	34.43	12.52	31.22	34.84	38.48	
Total nitrogen (N)		2.99	3.99	2.78	1.62	2.83	1.26	3.17	3.88	2.51	
Total carbonates (Cbt)		617	121	12	10	595	10	13	479	12	
Cation-exchange capacity (CEC)	mEq/kg	89.88	170.98	130.09	82.63	57.72	91.45	123.4	120.89	88.08	
<i>nirS</i>	log gene copy numbers/g dry soil	7.28	7.78	7.62	6.89	7.55	7.66	7.40	7.76	7.54	
<i>nirK</i>		8.77	8.78	9.21	8.61	8.80	8.87	9.03	9.24	9.00	
<i>AOA</i>		7.50	7.12	7.06	7.53	7.69	7.61	6.58	7.64	6.67	
<i>AOB</i>		7.03	7.09	7.36	7.27	7.52	7.13	7.11	7.01	6.82	
<i>nifH</i>		6.39	6.21	7.13	6.12	6.26	6.74	6.32	6.27	6.50	
<i>rRNA 16S</i>		10.88	10.97	11.07	10.71	11.01	11.01	10.94	10.99	10.82	

availability (Cantarel et al., 2015; Moreau et al., 2015, 2019). These modulations of soil N availability are strongly driven by the plant N demands and specific traits developed by plants to maximise N-resource acquisition (Boudsocq et al., 2012). Trait-based approaches have proved useful in explaining N-resource acquisition strategies developed by plants, from acquisitive (also called exploitative or competitive) to conservative strategies (Reich et al., 1999). Acquisitive plants exhibit some functional traits linked to plant performance such as high photosynthesis rates, high biomass, leaf or root density, leaf N or root N contents or high root exudation rates, which make them fast-growing species with high N capture capacities and utilisation efficiency (Grime, 2001). Consequently, ecosystem modifications caused by plant species on the N-cycle are largely the result of these strategies and functional traits (reviewed by Moreau et al., 2019). Invasive plants are known to express some of these traits linked to plant performance, such as, high biomasses (above- and belowground) and efficient N uptake (Ehrenfeld, 2010). Research demonstrates that invasive plants can alter the N-cycle in different ways, such as through the modification of the soil microbial community structure (Dassonville et al., 2008) or an increase in the abundance of microbial populations responsible for the N-cycle (McLeod et al., 2016). Moreover, invasive species tend to increase N fluxes (fixation, mineralisation, nitrification, denitrification) within terrestrial ecosystems (Hawkes et al., 2005; Liao et al., 2008; Ehrenfeld, 2010). Understanding the impact of invasive plants on C- or N-cycles relies on the characteristics of the plant traits but also on the environmental characteristics (invasibility) (Richardson and Pyšek, 2006). Indeed, environmental characteristics, such as soil biotic and abiotic properties, strongly influence plant functional traits, and the direction and magnitude of their effects on ecosystem processes (Mack and D'Antonio, 2003; Koutika et al., 2007; Scharfy et al., 2009). In the context of invasion and interactions between plant traits and the environment, the first question is: do plants induce generic effects on the N-

cycle or are these effects modulated as a function of soil type? The second question is: do invasive species known to alter the N-cycle have a stronger impact on the N-cycle than native species with the same N resource acquisition strategy? To answer these questions, this study focuses on two plants as models for invasive and native species: *Fallopia japonica* and *Dactylis glomerata*, respectively.

*Fallopia japonica* (Houtt.) Ronse Decraene var. *japonica*, belongs to the *Polygonaceae* family and is native to Japan and East Asia (Barney et al., 2006), where it grows in tetraploid, hexaploid or octoploid forms (Bailey et al., 2009) on riverbanks and disturbed areas (Maurel et al., 2013; Shimoda and Yamasaki, 2016). It is a perennial rhizomatous species belonging to the *Fallopia* spp. (Asian knotweed) species complex and is a well-studied invasive plant species. It was introduced to Europe in the 19th century by Phillippe von Siebold (Bailey and Conolly, 2000; Thiébaud et al., 2020) and is now widespread in North America and Europe. It is a vigorous herbaceous perennial that reproduces both clonally by regeneration from rhizomes and sexually with achenes. The *Fallopia* spp. complex is considered as one of the most invasive taxa in the world (Lowe et al., 2000). Some studies have been carried out on the effects of *Fallopia* spp. on ecosystems, and specifically on the N-cycle. *Fallopia* spp. is known for its acquisitive strategy and ability to modify soil nutrient pools, particularly for N mineral forms (Dassonville et al., 2008; Tharayil et al., 2013). Studies of *F. japonica* have observed seasonal variations in mineralisation in relation to the quality of the litter, which is known to be concentrated in secondary metabolites (Tharayil et al., 2013). Dassonville et al. (2011) have demonstrated that species of the *Fallopia* complex modulate nitrification and denitrification. These results have recently led to the identification of a particular strategy of biological denitrification inhibition (BDI) through the production and the release of secondary metabolites (Dassonville et al., 2011; Bardon et al., 2014). Through BDI, the plant increases N availability for its benefit, and consequently increases its competitive capacity (Bardon

et al., 2017). Among acquisitive plant species, *D. glomerata* (Cocksfoot grass) is well studied for its exploitative traits and its effect on C- and N-cycles (Cantarel et al., 2015; Legay et al., 2020a). It is a common and dominant perennial grass species, known to produce a large spectrum of root exudates which stimulate heterotrophic microbial enzymatic activities, and possessing acquisitive traits for N mineral forms associated with an acquisitive strategy (Grassein et al., 2015; Guyonnet et al., 2017, 2018).

The purpose of this study was to assess the impacts of the invasive *F. japonica* on the N-cycle and to compare the changes it caused with those caused by the native species *D. glomerata*. To achieve this, a mesocosm experiment was conducted by growing the invasive *F. japonica* and the native *D. glomerata* on nine different soils and measuring the impact of these plants on microbial activities in the N-cycle and the abundance of functional N-cycle genes.

## 2. Materials and methods

### 2.1. Soil characteristics and species studied

Nine soils from the Auvergne-Rhône-Alpes region in France were selected (Table S1) to ensure contrasted values on a large range of (a) biotic parameters (Table 1). The soils were mainly selected for their biotic traits as pertaining to their microbial community characteristics, such as, microbial enzymatic activity involved in the N-cycle (fixation, nitrification and denitrification), gene abundance in the total bacterial community (*rRNA 16S*) and functional gene abundances involved in the N-cycle (*nifH*, *AOA*, *AOB*, *nirK* and *nirS*). Soils were collected from the 0–30 cm topsoil layer (after removing plant litter) and sieved to 4 mm. The rates of clay, silt, sand, organic matter (OM), total nitrogen (N), total carbon (C), total carbonate (Cbt) and the cation exchange capacity (CEC) of the nine soils were measured by the Regional Agricultural Scientific Centre (CESAR, France; Table S2). Moisture content, pH, functional gene abundances involved in the N-cycle, total bacterial community abundance, microbial respiration and microbial enzymatic activity involved in the N-cycle and the N mineral forms (ammonium  $\text{NH}_4^+$  and nitrate  $\text{NO}_3^-$ ) were also recorded for each soil.

*Fallopia japonica* (*FJ*) was chosen as the invasive plant model, known for its acquisitive plant strategy. Rhizomes of a *FJ* population were collected from Le Chambon-Feugerolles site (Dassonville et al., 2011). They were selected for their similar diameter and node spacing. Each piece of rhizome was cleaned to remove soil before being cut into  $1 \text{ g} \pm 0.1$  equivalent pieces containing 1 node. *Dactylis glomerata* (*DG*) was chosen as the native plant model because it is also known for its acquisitive plant strategy. It is a perennial grass species classically found in areas invaded by *Fallopia* spp. *Dactylis glomerata* seeds were purchased from a seed company (Les Semences du Puy, Le Puy-en-Velay®, France).

*DG* and *FJ* were not present in the sampled soils before experiment. However, these two plants are very common in France.

### 2.2. Experimental design

A greenhouse experiment was conducted using 750 g of soil placed in pots (15 cm in diameter and 12 cm in depth). Each of the nine soils received three separate treatments. Each treatment comprised ten replicates (to ensure the regeneration of *FJ* rhizomes and the germination of *DG* seeds), for a total of 270 pots. In the first treatment, the soils remained unplanted (*UN*) – that is, the nine tested soils were without plant and root exudate influence. The second treatment consisted of planting two *DG* seeds. The third treatment was a planted treatment with a standardised fragment of *FJ* rhizome ( $1 \text{ g} \pm 0.1$ , containing a node). The pots were randomly arranged and moved randomly each week to avoid any localization effect. The pots were grown under controlled conditions (photoperiod = 16 h:8 h, light:dark; temperature 25 °C) in a glasshouse at the “Serre et Chambres Climatiques” platform (FR BioEEnVis UCBLyon1) for three months, and watered every three

days with removal of weeds. All the pots and plants received the same water treatment, without ever experiencing a period of severe drought (i.e. just normal soil drying between two watering). At the end, 135 pots ( $n = 5$ ) were harvested for microbial activity, gene abundances and plant measurements. At the time of harvest, *FJ* was in the vegetative growth stage (number of leaves  $\geq 6$ ) and *DG* was just beginning to flower.

### 2.3. Microbial activity measurements

Potential free-living nitrogen-fixation (FLNF) rates were measured using the acetylene ( $\text{C}_2\text{H}_2$ ) reduction technique (adapted from Patra et al., 2007 and Smercina et al., 2019). Ten grams (equivalent dry weight) of fresh soil were placed in a flask hermetically sealed with a rubber stopper. In each flask, air was removed and replaced with a He/ $\text{C}_2\text{H}_2$  mixture (90:10, v/v). Then, 1 % of this atmosphere was replaced by dioxygen ( $\text{O}_2$ ). A nutritive solution containing glucose (1 mg of C-glucose.g<sup>-1</sup> of dry soil), disodium malate (1 mg of C-disodium malate.g<sup>-1</sup> of dried soil) and distilled water was added through the rubber stopper using a syringe to ensure non-limiting amounts of carbon for FLNF activity. The amount of ethylene ( $\text{C}_2\text{H}_4$ ) during incubation at 28 °C was measured each hour during linear microbial growth phase (time chosen according to the kinetics observed for some samples). The slope of the linear regression was used to estimate the  $\text{N}_2$ -fixation with a conversion factor of 1/3  $\text{N}_2$  reduced per  $\text{C}_2\text{H}_2$  reduced ( $\mu\text{g C}_2\text{H}_4.\text{g}^{-1}$  dry soil.h<sup>-1</sup>). A gas chromatograph coupled to a flame ionisation detector (FID, Agilent 7890A, USA), fitted with an autosampler (SRA, France), measured  $\text{C}_2\text{H}_4$  concentrations.

Potential nitrification enzyme activity (NEA) was measured as  $\text{NO}_2^- + \text{NO}_3^-$  production (Dassonville et al., 2011). Three grams (equivalent dry weight) of fresh soil were placed in an airtight flask with a 21 %  $\text{O}_2$  atmosphere and supplemented with 30 ml of a water solution containing  $(\text{NH}_4)_2\text{SO}_4$  in order to provide 50  $\mu\text{g N}-(\text{NH}_4)_2\text{SO}_4$  per gram of dry soil and to ensure non-limiting amounts of  $\text{NH}_4^+$  for NEA activity. The flasks were incubated at 28 °C and shaken at 140 rpm. The soil suspension was sampled and filtered through a pore size of 0.22  $\mu\text{m}$  every 2 h for 10 h. The amount of  $\text{NO}_2^- + \text{NO}_3^-$  produced during incubation in samples for each kinetic sampling point was measured using a Smartchem 200 photometer (AMS Alliance, Villeneuve-la-Garenne, France). The slope of the linear regression was used to estimate the potential nitrification enzyme activity as a measure of  $\text{NO}_2^- + \text{NO}_3^-$  production ( $\mu\text{g N-NO}_2^- + \text{NO}_3^-.\text{g}^{-1}$  sol sec.h<sup>-1</sup>).

Potential denitrification enzyme activity (DEA) was measured as  $\text{N}_2\text{O}$  production using a method adapted from Dassonville et al. (2011). Ten grams (equivalent dry weight) of fresh soil were placed in a flask hermetically sealed with a rubber stopper. In each flask, air was removed and replaced with a He/ $\text{C}_2\text{H}_2$  mixture (90:10, v/v) to create anoxic conditions and inhibit  $\text{N}_2\text{O}$ -reductase. A nutritive solution containing glucose (0.5 mg of C-glucose.g<sup>-1</sup> of dry soil), glutamic acid (0.5 mg of C-glutamic acid.g<sup>-1</sup> of dried soil), potassium nitrate (50 mg of N- $\text{KNO}_3$ .g<sup>-1</sup> of dry soil) and distilled water was added through the rubber stopper using a syringe to ensure non-limiting amounts of carbon and  $\text{NO}_3^-$  for denitrification activity. The amount of  $\text{N}_2\text{O}$  during incubation at 28 °C was measured each hour for 6 h. The slope of the linear regression was used to estimate anaerobic respiration (denitrification) as the quantity of  $\text{N}_2\text{O}$  produced ( $\mu\text{g N-N}_2\text{O.g}^{-1}$  dry soil.h<sup>-1</sup>). A gas chromatograph coupled to a micro-katharometer detector (IGC-R3000; SRA instruments, Marcy L'Etoile, France) measured  $\text{N}_2\text{O}$  concentrations.

### 2.4. Soil nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) concentrations

Fresh soil samples (five grams equivalent of dry soil) were placed in plasma vials (150 ml). A volume of 0.01 M of  $\text{CaCl}_2$  solution (Houbat et al., 2000) was added after measuring the soil moisture. Soil suspensions were sealed with M® parafilm, incubated at 20 °C and shaken at 140 rpm for two hours at 10 °C. The suspensions were filtered through a

pore size of 0.22  $\mu\text{m}$ . A Smartchem 200 photometer (AMS Alliance, Villeneuve-la-Garenne, France) quantified the  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations.

## 2.5. Abundance of total bacteria and functional nitrogen-cycle genes

Microbial DNA was extracted from each soil sample using the NucleoSpin® Soil Kit (Macherey-Nagel GmbH & Co, Düren, Germany), following the manufacturer's protocol. DNA concentrations were determined with the Quant-iT™ PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Extracted DNA was stored at  $-20^\circ\text{C}$  until use. The relative abundance of bacterial *rRNA 16S* was measured as a proxy for the total bacterial community abundance, and the abundance of nitrogenase gene *nifH* as a proxy for the abundance of N-fixing microorganisms. In addition, the abundance of ammonia monooxygenase (*amoA*) genes *AOA* and *AOB* were measured as a proxy for the abundance of nitrifying archaea and bacteria respectively. Finally, the abundances of the nitrite reductase genes *nirS* and *nirK*, were measured as proxies for the abundance of denitrifying bacteria. Abundances were quantified based on the gene copy numbers present per gram of dry soil. Quantification was performed by quantitative real-time PCR (qPCR), using iTaq™ Universal SYBR® Green Supermix (Bio-Rad) as the fluorescent dye on a CFX Connect Real-Time PCR Detection System (Bio-Rad). All primer pairs and related qPCR conditions are detailed in Table S2.

The standard curves for *nifH*, *AOA*, *AOB*, *nirK* and *nirS* qPCR were generated by amplifying 10-fold dilutions ( $10^7$ – $10^2$ ), and for *rRNA 16S* qPCR by amplifying 10-fold dilutions ( $10^8$ – $10^2$ ) of a linearised plasmid. Melting curve analysis confirmed the specificity of amplification. Gene amplification efficiencies fell in the range of 85–99 %. R-squared ( $R^2$ ) values were always above 0.95. Possible inhibition of PCR was tested in advance and appropriate dilutions were chosen.

## 2.6. Plant growth traits

After plant sampling, aboveground and belowground parts of plants were separated. For *FJ*, a further separation was made into leaves, stems, roots and rhizomes. The belowground parts were manually removed from the soil and rinsed with water. The fresh mass and dry matter content (g MS) of the different organs were measured after lyophilisation (Cornelissen et al., 2003). Belowground biomass refer to roots for *DG* and roots and rhizome for *FJ*.

## 2.7. Statistical analyses

The effects of *FJ* and *DG* treatments compared to the *UN* treatment, named the plant effect, (Cantarel et al., 2020) on microbial enzyme activity (FLNF, NEA, DEA), on gene abundances (*nifH*, *AOA*, *AOB*, *nirK*, *nirS* and *rRNA 16S*) and on the mineral forms of N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) were measured according to the following formula:

$$\% \text{of } FJ \text{ or } DG \text{ effects compared to } UN = \frac{(\bar{X}_{FJ \text{ or } DG} - \bar{X}_{UN})}{\bar{X}_{UN}} \times 100$$

where,

- X: variable of interest for a given soil type and for a plant type (*FJ* or *DG*);
- $\bar{X}_{UN}$ : mean of the variable for the given unplanted soil;
- i: individual.

To test the statistical significance of the plant effect compared to *UN* ( $\mu = 0$ ), a multiple one-sample *t*-test was conducted. Negative and positive values indicate a negative or positive plant effect, respectively. To test the differences between plant effects, two-way ANOVA analysis was performed to find the effects of soil, plant treatment and their

interactions on the interest variables. Significant differences were determined using Tukey's HSD *post-hoc* tests. Therefore, the significant differences in plant treatment (i.e., difference between *DG* and *FJ*) will depend on their invasive status (i.e., native or invasive).

The  $\text{NO}_3^-$  mean value for each soil ( $n = 9$ ) under *FJ* and *DG* was regressed on the values for *UN*. As described in Dassonville et al. (2008), under the null hypothesis of no effect, all data points align on the line *FJ* or *DG* = *UN*. Deviation from this line, in the form of *FJ* or *DG* =  $b + aUN$ , indicates departure from the null hypothesis. Intercept (b) values > 0 and slope (a) values > 1 indicate a positive effect, (b) values < 0 and (a) values < 1 indicate a negative effect, and (b) values < 0 and (a) values > 1 or (b) > 0 and (a) < 1 indicate that the effect's direction depends on initial conditions. Conformity tests of intercept and slope were performed.

To test plant growth differences as a function of different soils, one-way ANOVA analysis was performed to find soil effects on the variables of interest. Significant differences were determined using Tukey's HSD *post-hoc* tests.

In order to test whether the plant effect of *DG* and *FJ* on microbial enzymatic activities (FLNF, NEA and DEA) was dependent on pre-trial soil parameters (hereafter called initial parameters), pairwise comparisons of Pearson's correlations between the plant effect on the microbial activities of the N cycle (i.e., FLNF, NEA and DEA) were tested on initial soil biotic and abiotic parameters (Table 1) for *FJ* and *DG*.

## 3. Results

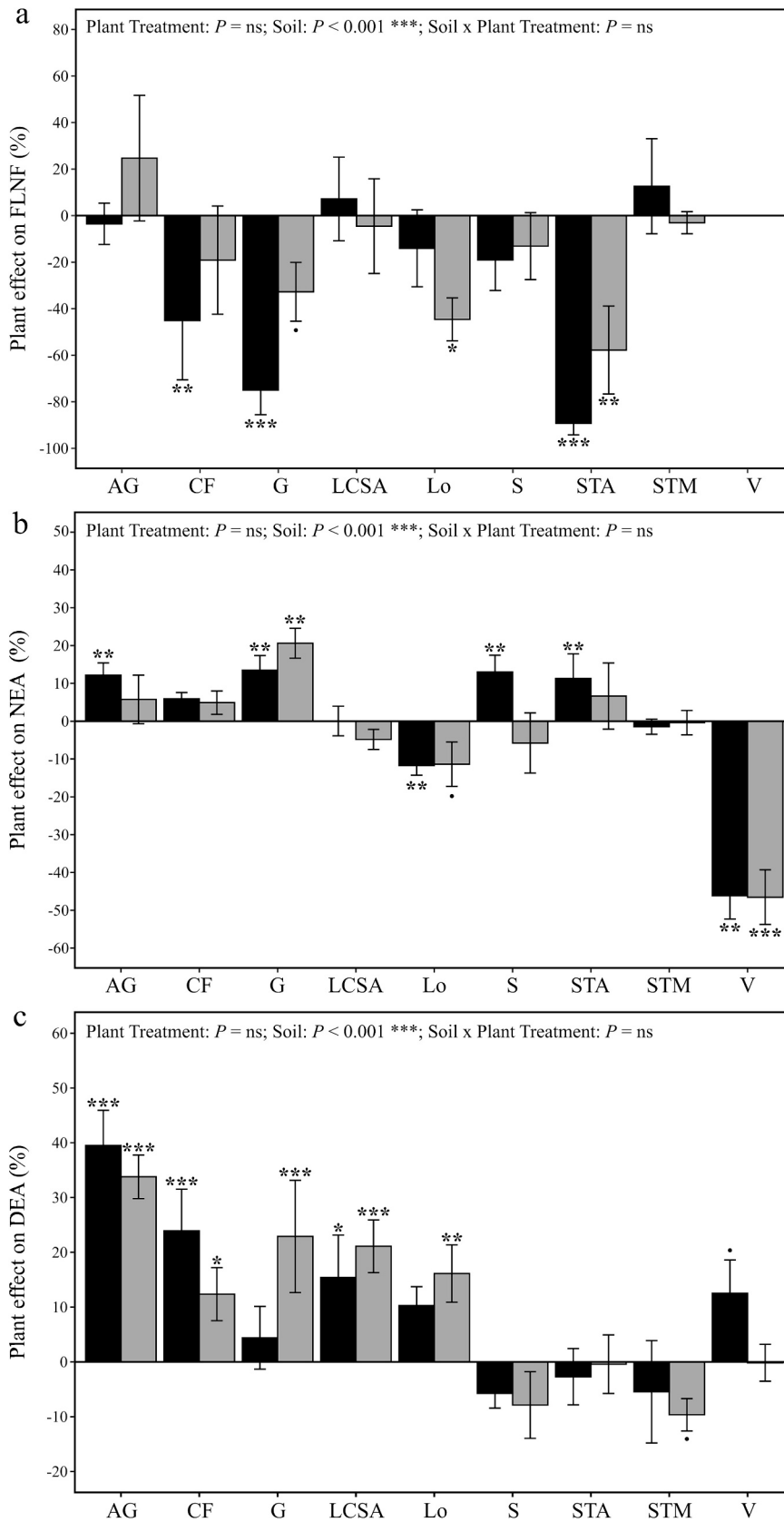
### 3.1. Plant modifications on microbial activities and N mineral forms

For free-living nitrogen-fixation (FLNF) rates, the plant effect is only dependent on soil ( $F = 6.17$ ,  $P < 0.001$ ), and no effect of invasive status was found (Fig. 1a, Table S3). Under *FJ*, FLNF rates decrease significantly in three soils (CF; G; STA - Fig. 1a). Under *DG*, FLNF rates decrease significantly in two soils (Lo and STA - Fig. 1a) and in soil G, there is also a statistical trend towards a decrease in FLNF. On soil V, the difference is very low or even equal to zero between all three treatments (*FJ*, *DG*, *UN*); it is therefore not possible to determine any plant effect.

The plant effect on nitrification (NEA) is impacted only by soil ( $F = 26.65$ ,  $P < 0.001$ ), and no effect of invasive status was found (Fig. 1b, Table S3). Under *FJ*, NEA increases significantly in four soils (AG; G; S; STA) and decreases significantly in two soils (Lo and V - Fig. 1b). Under *DG*, NEA increases significantly in G but also decreases significantly in V. Under *DG*, there is also a statistical trend towards a decrease in NEA in Lo.

The plant effect on denitrification (DEA) is only dependent on soil (*F*) and no effect of invasive status was found (Fig. 1c, Table S3). Under *FJ*, DEA increases significantly in three soils (AG; CF; LCSA - Fig. 1c) and increase marginally in V. Under *DG*, DEA increases significantly in five soils (AG; CF; G; LCSA; Lo) and decrease in STM.

Both soil ( $F = 20.27$ ,  $P < 0.001$ ) and plant treatment ( $F = 11.29$ ,  $P < 0.01$ ), without interaction ( $F = 1.40$ ,  $P = 0.21$ ), impact plant effect on  $\text{NO}_3^-$  concentrations (Fig. 2, Table S3). Under *FJ*, soil nitrate concentrations ( $\text{NO}_3^-$ ) decrease significantly in all soils, except in Lo soil, where the  $\text{NO}_3^-$  concentration does not differ significantly from that of *UN* (AG; CF; G; LCSA; S; STA; STM; V). Under *DG*,  $\text{NO}_3^-$  concentrations decrease significantly in six soils (CF; G; S; STA; STM; V). A stronger effect of the invasive status was found for the soil  $\text{NO}_3^-$  concentration, with a significant decrease under the *FJ* than under the *DG*. Under native *DG*,  $\text{NO}_3^-$  concentrations are linearly and positively related to those of *UN* (Fig. 2b). The linear regression differs from a theoretical regression ( $a = 1$  and  $b = 0$ ). This pattern is confirmed by the goodness of fit test for the slope of the regression, where the intercept is significantly greater than zero and the slope is significantly less than one (Fig. 2b). For invasive *FJ*, the regression is a second-degree polynomial (Fig. 2c) and the effect increases with increasing  $\text{NO}_3^-$  concentrations under *UN*. Under *FJ* and *DG*,  $\text{NH}_4^+$  concentrations were not significantly affected (data not



(caption on next page)

**Fig. 1.** *Fallopia japonica* (black) and *Dactylis glomerata* (gray) effect on potential microbial enzymatic activities involved in the N-cycle (a. FLNF (free-Living Nitrogen Fixation), b. NEA (nitrification), c. DEA (denitrification), in different soils, expressed as percent change from unplanted soil. Negative values indicate a negative effect of plant presence on microbial activities (values of microbial activity under plant treatment are lower than values for unplanted soil), and vice versa for positive values. Multiple one-sample *t*-tests were conducted to discover differences between planted and unplanted soils ( $\mu = 0$ ). The level of significance is indicated by asterisks: “.”,  $0.1 < P < 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . A two-way ANOVA analysis was performed to find soil, treatment and their interaction effects on the intensity of plant effect (percentage) on potential microbial enzyme activities compared to unplanted soil. Vertical bars indicate standard errors. Soil names are explained in Table S1.

shown).

In conclusion to this results section, plant effects on microbial activities (FLNF, NEA and DEA) are only soil dependent and no effect of invasive status was found. A significant negative effect of the invasive plant was only found for soil  $\text{NO}_3^-$  concentration. In addition, the native plant *DG* generally influenced microbial activities and soil N content over a wider range of soils than the invasive plant.

### 3.2. Plant modifications on functional gene abundances

For the FLNF-associated functional *nifH* gene abundance, soil ( $F = 5.25$ ,  $P < 0.001$ ), plant treatment ( $F = 6.68$ ,  $P < 0.05$ ) and the interaction ( $F = 4.55$ ,  $P < 0.001$ ) impact plant effect (Fig. 3a, Table S3). A stronger effect of the native status was found with a significant increase by *DG* when compared to *FJ* in soils LCSA and STA (Fig. 3a). Under the invasive *FJ*, FLNF-associated functional *nifH* gene abundances are not significantly affected. Conversely, under the native *DG*, *nifH* abundances increase significantly in three soils (CF; LCSA; STA).

For the NEA-associated functional AOA gene abundance, the soil x plant treatment interaction ( $F = 5.21$ ,  $P < 0.001$ ) has an effect on the plant effect (Fig. 3b, Table S3). In soil G, native status has a stronger negative effect on AOA abundances than invasive, but the opposite is true for soil STA, with a stronger positive effect of native than invasive species (Fig. 3b). Under *FJ*, NEA-associated AOA functional gene abundances are not significantly affected. Conversely, under *DG*, AOA abundances decrease significantly in three soils (AG; G; S) and increase in STA.

For the NEA-associated functional AOB abundance, the soil ( $F = 6.06$ ,  $P < 0.001$ ) and the interaction between plant treatments and soil ( $F = 9.30$ ,  $P < 0.001$ ) have an effect on the plant effect (Fig. 3c, Table S3). The same pattern was found for AOB than AOA abundances, a decrease of AOB abundances in soil G under native species and an increase in soil STA (Fig. 3c). Under *FJ*, NEA-associated functional AOB gene abundances decrease significantly in two soils (AG and STA) and increase in V. Under *DG*, AOB abundances decrease significantly in G and marginally in AG. AOB abundances increase in two soils (STA and V).

For the DEA-associated functional *nirK* gene abundance, plant treatment ( $F = 6.44$ ,  $P < 0.05$ ), soil ( $F = 4.91$ ,  $P < 0.001$ ) and soil x plant treatment interactions ( $F = 4.93$ ,  $P < 0.001$ ) influence the plant effect (Fig. 3d, Table S3). In general, native status has a stronger positive effect on *nirK* abundance than invasive, but this result is largely driven by STA soil with an increase in *nirK* abundance under *DG* six times higher than that found in the other soils (Fig. 3d). Under *FJ*, *nirK* abundances decrease significantly in AG and marginally in STA. Under *DG*, the *nirK* abundance increases significantly only in STA.

For DEA-associated functional *nirS* gene abundances, soil ( $F = 4.47$ ,  $P < 0.001$ ) and soil x plant treatment interactions ( $F = 5.92$ ,  $P < 0.001$ ) impact plant effect (Fig. 3e, Table S3). In soil G, native status has a stronger negative effect on *nirS* abundances than invasive, but the opposite is true for STA. Under *FJ*, *nirS* abundances decrease significantly in V and marginally in STA. The *nirS* abundances increase in CF. Under *DG*, *nirS* abundances decrease significantly in G and increase in CF and STA.

For *rRNA 16S* gene abundances, plant treatment ( $F = 6.48$ ,  $P < 0.05$ ), soil ( $F = 4.52$ ,  $P < 0.001$ ) and soil x plant treatment interaction ( $F = 5.76$ ,  $P < 0.001$ ) impact plant effect (Fig. 3f, Table S3). In soil G, *rRNA 16S* abundances decrease more significantly under native than under

invasive species, but the opposite is true for STA. Under *FJ*, *rRNA 16S* are not significantly affected. Under *DG*, *rRNA 16S* abundances increase significantly in four soils (CF; LCSA; STA; V) and decrease in two soils (AG and G).

In conclusion to this results section, stronger impacts of the native *DG* on microbial gene abundances are generally found compared to the invasive *FJ*.

### 3.3. Plant growth

Soil affected *FJ* and *DG* aboveground and belowground biomasses, and total biomass. The aboveground biomass of *FJ* was significantly greater in soil CF than in soils AG, LCSA, Lo, S, STA, STM and V, and greater in soil G than in soils AG, LCSA, Lo and STM (Fig. 4a). Its belowground biomass was significantly greater in soil CF than in the eight other soils, and greater for soil STA than for STM (Fig. 4c). The total biomass of *FJ* was significantly greater in soil CF than in soils AG, G, LCSA, Lo, S, STA, STM and V, and greater in soil STA than in soils AG and STM (Fig. 4e). The *DG* aboveground biomass was significantly greater in soil STA than in soils LCSA, STM and V, and greater in soils S and Lo than in soils STM and V (Fig. 4b). *DG* belowground biomass was significantly greater in STA than in AG, G, LCSA, Lo, S, STM and V, and greater in CF than in AG, LCSA, Lo, S, STM and V (Fig. 4d). Its total biomass was significantly greater in soils CF and STA than in soils AG, LCSA, Lo, S, STM and V, and greater in soil G than in soils LCSA, STM and V (Fig. 4f). In addition, *DG* total biomass was significantly greater in soil S than in soil STM (Fig. 4f).

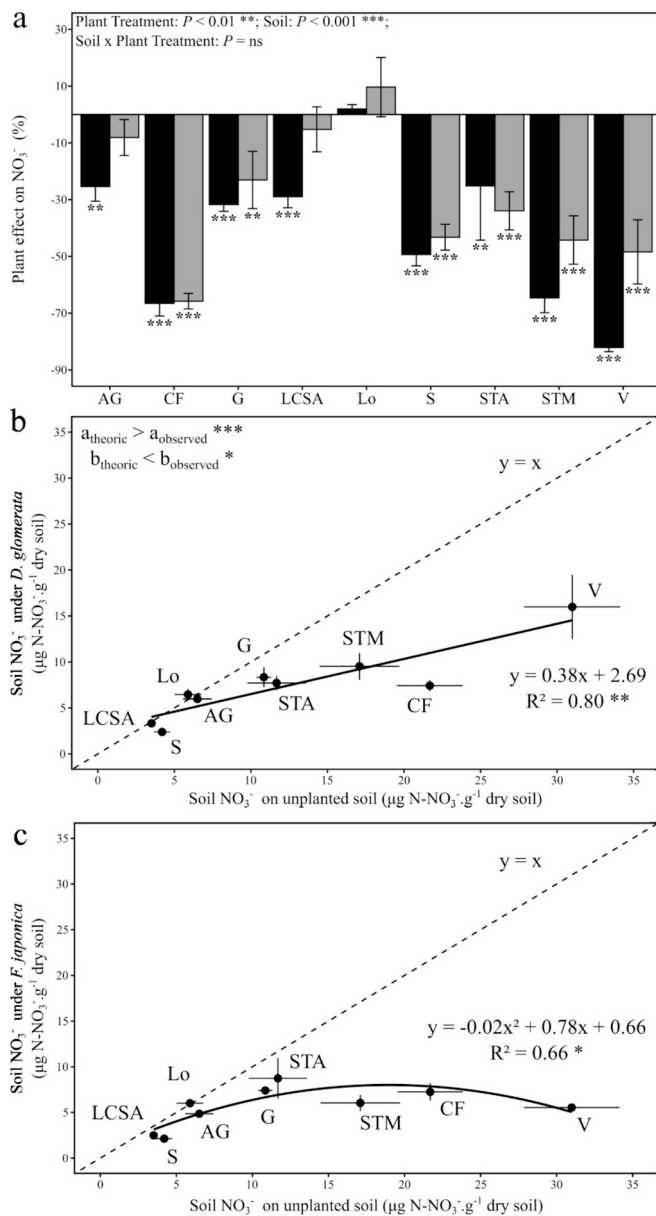
In conclusion to this results section, *FJ* and *DG* above-, belowground and total biomasses are largely influenced by soils.

### 3.4. Influence of initial soil parameters on *DG* and *FJ* effects on microbial enzyme activities

For the plant effect on FLNF, *FJ* is correlated with 11 initial soil parameters, while *DG* is correlated with 8 (Table 2). Five initial soil parameters are common between *DG* and *FJ* with positive (i.e., NEA, AOA, pH, total carbonates) or negative (i.e., soil  $\text{NO}_3^-$  concentration) correlations. *FJ* is also sensitive to other soil initial parameters, always with negative correlations between *FJ* effect on FLNF (i.e., *nifH*, soil  $\text{NH}_4^+$  concentration, moisture, organic matter, cation exchange capacity). The *DG* effect was specifically positively correlated with initial DEA and silt and negatively correlated with initial sand quantity (Table 2).

For the plant effect on NEA, *FJ* is correlated with 9 initial soil parameters, while *DG* is correlated with 13 (Table 2). Six initial soil parameters are common between *DG* and *FJ* with positive (i.e., *nifH*, soil  $\text{NH}_4^+$  concentration, cation exchange capacity) or negative (i.e., AOA, AOB, total carbonates) correlations. *FJ* responded to other initial soil parameters, with negative correlations with initial NEA and pH, and a positive correlation with initial moisture. The effect of *DG* on NEA is highly sensitive to soil abiotic parameters, particularly soil texture, as correlations were found with clay, silt, sand, organic matter, total carbon, and nitrogen (Table 2).

For the plant effect on DEA, *FJ* is correlated with 6 initial soil parameters, while *DG* is correlated with 7 (Table 2). Only three initial soil parameters are common between *DG* and *FJ* with positive (i.e., FLNF) or negative (i.e., *nirK*, *nirS*) correlations. *FJ* responded to other initial soil parameters, with negative correlations with initial *nifH* abundance and clay presence, and a positive correlation with initial moisture, whereas



**Fig. 2.** *Fallopia japonica* (black) and *Dactylis glomerata* (gray) effect on soil  $NO_3^-$  concentration, in different soils, expressed as percent change from unplanted soil (a) and soil  $NO_3^-$  concentration under plants (b. *DG* and c. *FJ*) as a function of soil  $NO_3^-$  concentration in unplanted soil. (a) Negative values indicate a negative effect of plant presence on gene abundance (values of gene abundance under plant treatment are lower than values on unplanted soil), and vice versa for positive values. Multiple one-sample t-tests were conducted to discover differences between planted and unplanted soils ( $\mu = 0$ ). The level of significance is indicated by asterisks: “.”,  $0.1 < P < 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . A two-way ANOVA analysis was performed to find soil, treatment and their interaction effects on the intensity of plant effect (percentage) on gene abundance compared to unplanted soil. Vertical bars indicate standard errors. (b and c) The dashed line ( $X = Y$ ) shows no impact of plant on soil  $NO_3^-$  concentrations compared to unplanted soil. Points above the line indicate increase under plants and points below the line a depletion under plants. The black solid line represents the regression line. Vertical and horizontal bars indicate standard errors. Soil names are explained in Table S1.

*DG* responded positively to initial NEA, pH, silt, and total carbonates (Table 2).

In conclusion to this results section, the influence of initial abiotic and biotic soil factors are common to both species, except for nitrification. For this activity, the native effects are correlated with initial abiotic

factors (clay, silt, sand, organic matter, total carbon and nitrogen content), whereas invasive effects are not.

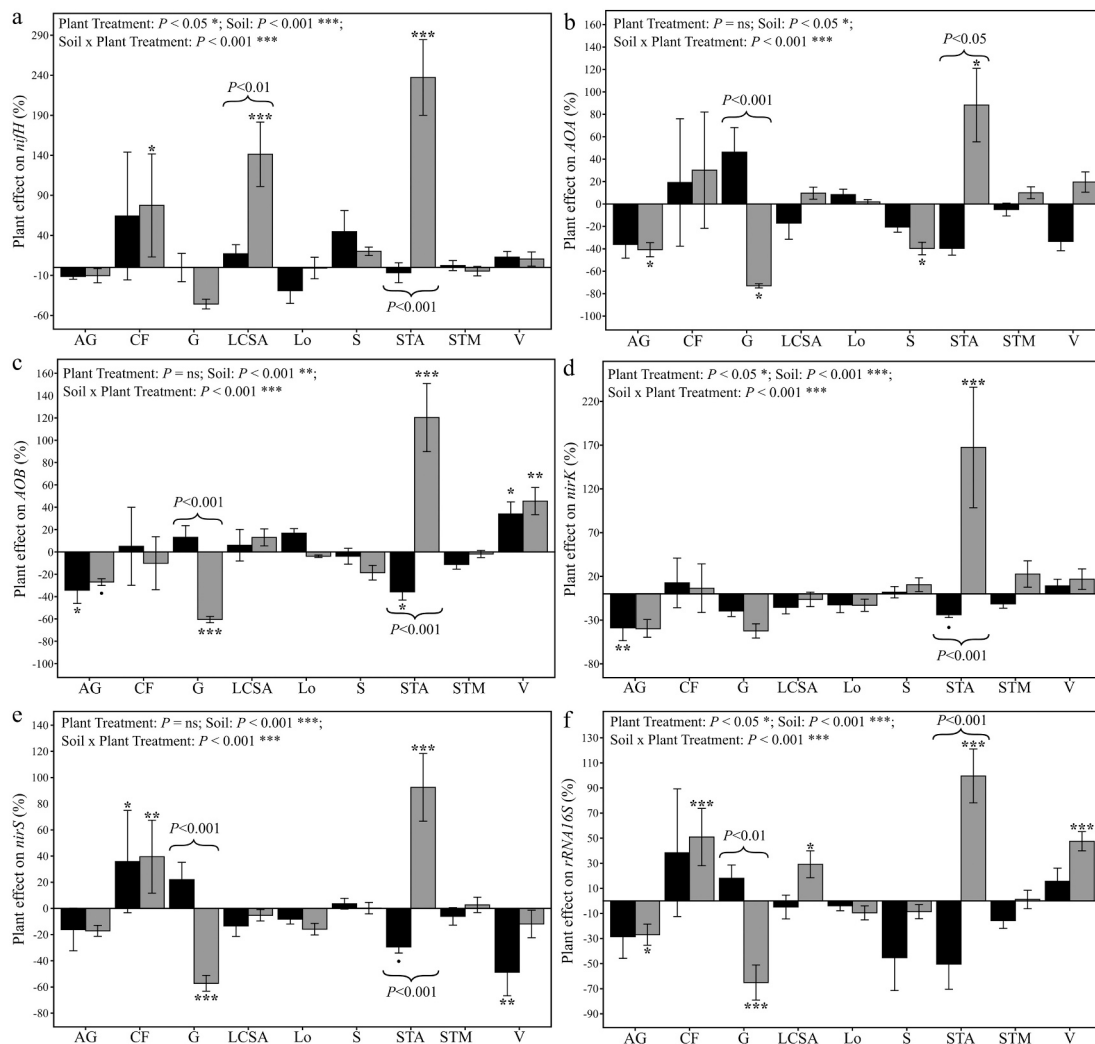
#### 4. Discussion

The present study aimed to compare the effect of a native plant and an invasive one with similar N-resource strategy (i.e., acquisitive strategy) on soil N-cycle and its associated soil microbial abundances in nine different soil types and to compare the results with those of the respective unplanted soils. The N-cycle plays a key role in ecosystem functioning, especially for plant growth (LeBauer and Treseder, 2008). The influence of plants on these microbial processes is of the utmost importance. This study covers almost the entire range of microbial processes in the N-cycle, from abundance to microbial activity and enzymatic by-products, giving us a better understanding of the potential impacts of invasive and native plants on the N-cycle functioning.

##### 4.1. Invasive and native status impact on N-cycle functioning and plant growth

The *FJ* and *DG* plants strongly modulate microbial N-cycle enzymatic activity (i.e., increase or decrease it when compared to the unplanted soils). Though the two plants do not modulate microbial enzymatic activity with the same intensity, the plant effect on N-microbial activities is only dependent on soil type and never on the invasive or native status. On free-living nitrogen-fixation (FLNF) rates, the two plants, *FJ* and *DG*, have few effects when compared to those in unplanted soil (i.e. no effect in six out of nine soils). These results suggested that invasive or native plant status have only a limited impact on FLNF in the studied soils, despite the fact that FLNF appears as beneficial for plants (Canbolat et al., 2006). Some free N-fixing bacteria are known to promote plant growth and are called PGPR (Jha and Saraf, 2015). The influence of plants on FLNF is still poorly understood, and soil parameters appear to be important factors in controlling FLNF (Smircina et al., 2019), such as the quality and/or quantity of soil C and N, pH, water and oxygen concentrations, or the soil nutrient pool (Smircina et al., 2019). For nitrification, both *DG* and *FJ* influence NEA positively or negatively, with an impact on a wider range of soils for the invasive species, though with no effect of invasive status was found. The *FJ* plant therefore modulates nitrification in six soils as opposed to only three for *DG*, suggesting that *FJ*'s ability to control this step of the N-cycle is greater than that of *DG*'s and could represent an advantage in nitrogen resource acquisition. Nitrification rates can increase under plants, and it is generally the case for *FJ*. Invasive plants are known to predominantly increase nitrification rates in plant and litter biomass, plant and litter N concentration, and litter C:N ratios (Liao et al., 2008). Nevertheless, the experimental protocol in this study excluded an analysis of litter effect on NEA. The present results show that *DG* and *FJ* can also decrease NEA with the same intensity and on the same soils, suggesting that certain environmental factors could condition plant responses, whatever the species. Nevertheless, some plant traits relative to soil resource acquisition strategies, such as plant affinity for  $NH_4^+$  or root prospecting, are linked to nitrification rates (Cantarel et al., 2015; Abalos et al., 2018). Moreau et al. (2015, 2019) suggest that plants' high capacity to acquire N mineral forms can limit the activity of microorganisms in the N-cycle. The production and release of secondary metabolites by roots of native or agricultural species can also inhibit NEA (Subbarao et al., 2007; Zhang et al., 2022). Denitrification (DEA), as NEA, is an important microbial enzyme activity in relation to soil N losses (Radersma and Smit, 2011). Compared to unplanted soils, two types of response were observed under plant treatment, either stimulation of DEA or no effect on DEA. However, no effect on invasive or native status was found. Several studies have shown that denitrification increases under some plants, invasive (Zou et al., 2006; Gao et al., 2019; Zhang et al., 2020) and native (Allred and Baines, 2016; Guyonnet et al., 2017; Abalos et al., 2018). Increased anaerobic microbial respiration could result from



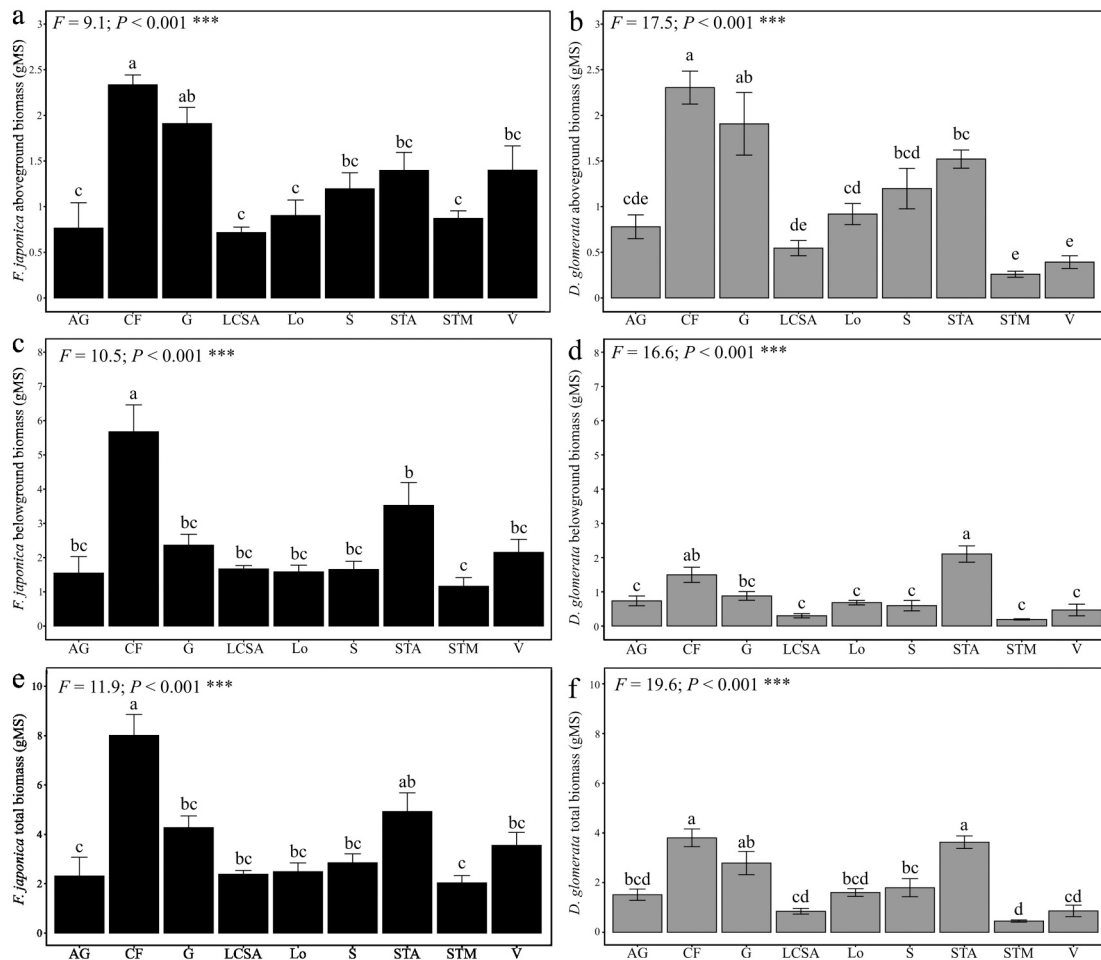


**Fig. 3.** *Fallopia japonica* (black) and *Dactylis glomerata* (gray) effect on functional gene abundances involved in the N-cycle (a. *nifH*, b. AOA, c. AOB, d. *nirK*, e. *nirS*) and on total bacterial community gene abundance (f. *rRNA 16S*), in different soils, expressed as percent change from unplanted soil. Negative values indicate a negative effect of plant presence on gene abundance (values of gene abundance under plant treatment are lower than values for unplanted soil), and vice versa for positive values. Multiple one-sample t-tests were conducted to discover differences between planted and unplanted soils ( $\mu = 0$ ). The level of significance is indicated by asterisks: “.”,  $0.1 < P < 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . A two-way ANOVA analysis was performed to find soil, treatment and their interaction effects on the intensity of plant effect (percentage) on gene abundance compared to unplanted soil. Vertical bars indicate standard errors. Soil names are explained in Table S1.

higher root exudation rates, especially of C compounds (the rhizosphere effect) (Henry et al., 2008). High root exudation rates characterise plants with an acquisitive strategy for soil resources and lead to an increase in denitrification (Guyonnet et al., 2018). However, plant stimulation on denitrification is still debated and seems to be confined to pockets of air-filled porosities in the soil with an  $O_2$  content below 10%–12% (Prade and Trollenier, 1988; Moreau et al., 2019). On the other hand, the rhizosphere effect of both plants for some soils was either absent or counterbalanced by a DEA inhibition effect and/or a strong competition for nitrate between plants and a denitrifying community. It has been demonstrated that *Fallopia* spp. can inhibit denitrification through the release of procyanidins in root exudates, a phenomenon called biological denitrification inhibition (BDI) (Bardon et al., 2014, 2016). For *FJ*, a BDI strategy in certain soils could counterbalance the rhizosphere effect. However, *DG* is known to not produce denitrification inhibitors (Guyonnet et al., 2018), but for this species, efficient N uptake through the root, and particularly of  $NO_3^-$ , can negatively affect DEA by reducing the abundance of denitrifiers (Grassein et al., 2014).

Unlike N-cycle microbial activities, soil  $NO_3^-$  concentration and the N-cycle gene abundances are strongly influenced by the plant invasive or

native status. In this study, a stronger effect of the invasive status was found for the soil  $NO_3^-$  concentration (i.e. without interaction effects between soil type and plant status), with a significant decrease under the *FJ* than under the *DG*. The *Fallopia* species complex can modify N mineral pools (Tharayil et al., 2013; Dassoiville et al., 2008) and immobilise these N-forms via storage in its rhizome (Aguilera et al., 2010). In addition, *FJ* has developed a particular  $NO_3^-$  acquisition strategy, BDI, which increases  $NO_3^-$  availability for its benefit, stimulates root system growth, and consequently increases its capacity to acquire N resources (Bardon et al., 2014, 2017). The *FJ* rhizome system could act as a N-uptake pathway, with the potential to exploit more a volume of soil than the root system (Brooker et al., 1999). The effect of invasive status on these results must be qualified by the difference in morphological traits between these two plants which belong to different and distant botanical families (i.e. *Poaceae* in Monocotyledons for *DG* and *Polygonaceae* in Eudicotyledons for *FJ*), and by a conservation strategy specific to rhizomatous plants such as *FJ*, which is not active for *DG*. The soil  $NH_4^+$  pool is not influenced by *FJ* and *DG* when compared to unplanted soil rates. Ammonium ( $NH_4^+$ ) is less mobile than  $NO_3^-$  in soils and therefore usually less available for plant N uptake (Courty et al., 2015). In



**Fig. 4.** *Fallopia japonica* (black) and *Dactylis glomerata* (gray) growth traits, expressed in g of dry mass (gMS) (a and b: aboveground biomass; c and d: belowground biomass; e and f: total biomass) in different soils. A one-way ANOVA analysis was performed to identify a soil effect on the plant traits. If significant soil effect was found, post-hoc tests were conducted to discover differences among soils through a post-hoc Tukey HSD test. Different letters indicate a significant difference ( $P < 0.05$ ). Vertical bars indicate standard errors. Soil names are explained in Table S1.

addition, the present study reveals that the native plant impact on the abundances of the N-cycle microbial community is greater (i.e., affects a wider range of genes) than that invasive plant and extends to the total bacterial community. The *DG* plant significantly modifies the abundance of all studied functional genes (*nifH*, *AOA*, *AOB*, *nirK*, *nirS* and *rRNA 16S*). In contrast, *FJ* only modifies significantly the abundance of the functional denitrification genes (*nirK*, *nirS*) and the abundance of the functional nitrification bacterial gene (*AOB*). It appears that *FJ*'s strategy for controlling the N-cycle is mainly linked to controlling and competing with the denitrifying community for  $\text{NO}_3^-$  acquisition. Acquisitive plant species are known to modulate nitrifying and denitrifying abundances in their rhizosphere (Michalet et al., 2013; Moreau et al., 2015; Thion et al., 2016). Some studies pertaining to invasive plant species have also demonstrated modulations of functional N-cycle gene abundances (Kourtev et al., 2003; Hawkes et al., 2005; Xu et al., 2012). Once again, soil type is a factor that strongly determines the plant response, particularly for the native plant, with strong opposite effects on G and STA soils between native and invasive plants. *DG* generally decreases some functional gene abundances for G soil (*AOA*, *AOB*, *nirS* and *rRNA 16S*), while increasing all studied gene abundances for STA. The G and STA soils are quite similar in initial gene abundance, suggesting that this opposite effect of the native plant may be directly related to microbial communities and their diversity. Surprisingly, both the invasive and the native plant reach their highest total biomass on this soil. This suggests that even though the two plants show opposite behaviour in terms of

impact on functional gene abundance, they have similar growth on this soil. But, the two plants also have the highest growth on the CF soil, while the effects of the plants on the N-cycle functioning are the same. These two soils are different in terms of initial soil conditions (i.e., higher values of organic matter, carbon and carbonates levels in the CF soil than the STA soil), highlighting once again the importance of soil type for the plant behaviour. At present, it remains complicated to decipher the importance of each biotic and abiotic soil parameter, and it seems that microbial diversity also plays a role. Further studies on these three soils are needed to understand the impact of (a)biotic factors on plant behaviour.

#### 4.2. Influence of initial soil parameters on effects of native and invasive plants on N-cycle functioning

The influence of invasive plants on ecosystem functioning is known to be modulated by environmental conditions (Ehrenfeld, 2010; Vilà et al., 2011; Pyšek et al., 2012), including effects on the N-cycle (Liao et al., 2008). This study evaluated the impact of plants on the N-cycle using nine different soils as a means to introduce different environmental parameters into the control of the N-cycle by plants and to be able to generalise or not these impacts. Results show that across the nine soils, *FJ* and *DG* have strong impact on N-cycle functioning often similar as for microbial activities and sometimes drastically different as for soil  $\text{NO}_3^-$  concentration and functional gene abundances. This confirms that

**Table 2**

Influence of initial soil parameters (a: the initial biotic parameters; b: the initial soil concentrations of N mineral forms and c: the initial soil abiotic parameters) on *Fallopia japonica* and *Dactylis glomerata* effects on microbial enzyme activities (FLNF (free-Living Nitrogen Fixation), NEA (nitrification) and DEA (denitrification)). Here were shown pairwise comparisons of Pearson's correlation (r).

	Plant effect on FLNF				Plant effect on NEA				Plant effect on DEA			
	<i>Fallopia japonica</i>		<i>Dactylis glomerata</i>		<i>Fallopia japonica</i>		<i>Dactylis glomerata</i>		<i>Fallopia japonica</i>		<i>Dactylis glomerata</i>	
a) Initial soil biotic parameters (N-microbial activities and associated gene abundances)												
FLNF	ns		ns		ns		ns		0.35	*	0.22	*
<i>nifH</i>	-0.36	*	ns		0.37	*	0.43	***	-0.18	**	ns	
NEA	0.46	***	0.21	**	-0.44	***	ns		ns		0.29	*
AOA	0.64	***	0.22	*	-0.49	***	-0.51	***	ns		ns	
AOB	ns		ns		-0.42	***	-0.16	**	ns		ns	
DEA	ns		0.15	*	ns		ns		ns		ns	
<i>nirK</i>	ns		ns		ns		0.33	***	-0.46	***	-0.32	*
<i>nirS</i>	ns		ns		ns		ns		-0.52	***	-0.51	**
b) Initial soil concentrations of N mineral forms												
NO <sub>3</sub> <sup>-</sup> concentration	-0.39	**	-0.25	**	ns		ns		ns		ns	
NH <sub>4</sub> <sup>+</sup> concentration	-0.46	***	ns		0.54	***	0.43	***	ns		ns	
c) Initial soil abiotic parameters												
pH	0.54	***	0.34	***	-0.32	***	ns		ns		0.19	*
Moisture	-0.23	**	ns		0.17	*	ns		0.47	***	ns	
Clay	ns		ns		ns		0.33	***	-0.25	*	ns	
Silt	ns		0.22	*	ns		0.25	***	ns		0.30	*
Sand	ns		-0.24	*	ns		-0.34	***	ns		ns	
Total Carbon	-0.24	*	ns		ns		0.33	**	ns		ns	
Total Nitrogen	ns		ns		ns		0.23	**	ns		ns	
Organic matter	-0.24	*	ns		ns		0.33	**	ns		ns	
Total carbonates	0.42	**	0.18	*	-0.36	***	-0.21	**	ns		0.19	*
Cation exchange capacity	-0.37	***	ns		0.34	***	0.44	***	ns		ns	

Pearson Correlation coefficient (r). Significance levels (ns = not significant, \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001).

plant functional traits and consequently their effects on soil properties and functions are modulated by soil parameters (Cantarel et al., 2015; Moreau et al., 2015). It is therefore illusory to attempt to characterise a plant by its effects on the ecosystems without specifying the biotic and abiotic contexts. This study found that some initial abiotic and biotic soil factors strongly influence the plant effects on microbial activities. As found in a recent study (Béraud et al., 2024), biotic factors are also relevant to explain plant modulation of soil microbial activities. Here, the results showed that the influence of soil parameters is strongly dependent on the microbial activities studied (Zifcakova, 2020). Many of these correlations are common to both species. However, for nitrification, the native effects are correlated with initial abiotic factors such as clay, silt, sand, organic matter, total carbon and nitrogen, whereas the invasive are not. These results could be explained by phylogenetic differences between *DG* and *FJ*, respectively *Poaceae* and *Polygonaceae* families. The *Poaceae* are known to be highly capable of modulating microbial activities, such as nitrification with the biological inhibition nitrification (BNI, Subbarao et al., 2007), which is present in several genera, as *Brachiaria*, *Leymus*, *Panicum*, *Pennisetum* and *Sorghum*. Moreover, this BNI is carried out by a wide range of biological inhibitors, suggesting effectiveness in many ecosystems, and therefore, on a wide range of soil parameters, which could have favoured the *Poaceae* ability, like *DG*, to better perceive soil abiotic factors.

The effect of an introduced plant is circumstantial. It depends on both the biological traits of the introduced species and the sensitivity of the receiving ecosystem to the invasion (Ehrenfeld, 2010; Vilà et al., 2011; Pyšek et al., 2012). The present experimental design (i.e., the use of nine soils) facilitates understanding this circumstantiality and partly quantifying the role of the receiving ecosystem. Both species affect the N cycle, with the native plant having a stronger effect on microbial community abundances. The many studies carried out on invasive *Fallopia* spp. and their strategies for acquiring N led us to envisage and generalise a broader and strong effective impact on ecosystem functioning. To date, no study has compared this impact with that of a native plant displaying similar N resource acquisition traits. The present results also highlight

the possible presence of a bias due to the social representation of invasive species (Cassini, 2020), leading to a presumption of guilt for non-native plants (Guiasu and Tindale, 2018). Indeed, in Japan, from where it is partly native, *FJ* is a very common plant, even dominant in certain ecosystems. Despite its widespread distribution and dominance (Ito et al., 1982; Nashiki et al., 1986), it is not perceived negatively. Based on this observation, Shimoda and Yamasaki (2016) investigated the differences in perception of this species between Japan, and Europe and North America, where it is considered one of the worst invasive alien species. In Europe, conversely, *DG* is widely used as a perennial grass species model (Cantarel et al., 2015; Guyonnet et al., 2018; Legay et al., 2020a, 2020b). Some of these studies deal with the influence of *DG* on the N-cycle, without however being biased in their conclusions against cocksfoot grass. Nonetheless, *DG* is considered an invasive species in North America (MacDougall and Turkington, 2005; Luong et al., 2023) and, in Japan, it is even listed as one of the 16 most invasive ones (Muranaka et al., 2005). The present results confirm that invasion ecology is subject to perception (Kapitza et al., 2019) and raise the question of choosing the right control to determine the effects of an introduced plant on an ecosystem. Hulme et al. (2013) suggested studying species with negligible impacts or species with known ecological impacts, to understand factors that influence plant community, extend the range of ecological variables examined or increase the number of study locations. This study suggests the use of native controls that are likely to stand up to comparison, i.e., with the same morphological architecture and/or similar strategies (growth, reproduction, etc.). The present study shows that *DG* is one of these native controls for the N-cycle.

## 5. Conclusions

Here, the plant effects on microbial enzymatic activities of the soil N-cycle do not allow to distinguish them in terms of their native status or not in the ecosystem. In contrast, the plants differ in their influence on microbial abundances, with widespread control of the microbial

community in favour of the native plant (i.e., *DG*). The native plant generally influenced microbial parameters over a wider range of soils than the invasive plant. Finally, plant responses and plant traits were strongly soil dependent. Without questioning the influence of plant species on ecosystem functioning, this study suggests that introduced species should benefit from a presumption of innocence, at least until subject to a detailed comparative analysis against plants that can withstand the comparison.

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### CRedit authorship contribution statement

**C. Béraud:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **A.A.M. Cantarel:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **J. Gervais:** Methodology, Formal analysis. **C. Creuzé des Châtelliers:** Methodology, Formal analysis. **A. Delort:** Methodology, Formal analysis. **C. Boisselet:** Methodology, Formal analysis. **S. Poussineau:** Methodology, Formal analysis. **E. Lacroix:** Methodology, Formal analysis. **F. Piola:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### References

Abalos, D., Van Groenigen, J.W., De Deyn, G.B., 2018. What plant functional traits can reduce nitrous oxide emissions from intensively managed grasslands? *Glob. Chang. Biol.* 24, e248–e258. <https://doi.org/10.1111/gcb.13827>.

Aguilera, A.G., Alpert, P., Dukes, J.S., Harrington, R., 2010. Impacts of the invasive plant *Fallopia japonica* (Houtt.) on plant communities and ecosystem processes. *Biol. Invasions* 12, 1243–1252. <https://doi.org/10.1007/s10530-009-9543-z>.

Ahmad, R., Khuroo, A.A., Hamid, M., Rashid, I., 2019. Plant invasion alters the physicochemical dynamics of soil system: insights from invasive *Leucanthemum vulgare* in the Indian Himalaya. *Environ. Monit. Assess.* 191, 792. <https://doi.org/10.1007/s10661-019-7683-x>.

Allred, M., Baines, S.B., 2016. Effects of wetland plants on denitrification rates: a meta-analysis. *Ecol. Appl.* 26, 676–685. <https://doi.org/10.1890/14-1525>.

Araya, T., Mlahlwa, A.V., Elbasit, M.A.M.A., Newete, S.W., 2022. The impact of *Tamarix* invasion on the soil physicochemical properties. *Sci. Rep.* 12, 5750. <https://doi.org/10.1038/s41598-022-09797-3>.

Bailey, J.P., Conolly, A.P., 2000. Prize-winners to pariahs - a history of Japanese knotweed s.l. (*Polygonaceae*) in the British Isles. *Watsonia* 23, 93–110.

Bailey, J.P., Bimová, K., Mandák, B., 2009. Asexual spread versus sexual reproduction and evolution in Japanese Knotweed s.l. sets the stage for the “Battle of the Clones”. *Biol. Invasions* 11, 1189–1203. <https://doi.org/10.1007/s10530-008-9381-4>.

Bardon, C., Piola, F., Bellvert, F., Haichar, F.E.Z., Comte, G., Meiffren, G., Pommier, T., Puijalon, S., Tsafack, N., Poly, F., 2014. Evidence for biological denitrification inhibition (BDI) by plant secondary metabolites. *New Phytol.* 204, 620–630. <https://doi.org/10.1111/nph.12944>.

Bardon, C., Poly, F., Piola, F., Pancton, M., Comte, G., Meiffren, G., Haichar, F.E.Z., 2016. Mechanism of biological denitrification inhibition: procyanidins induce an allosteric transition of the membrane-bound nitrate reductase through membrane alteration. *FEMS Microbiol. Ecol.* 92, fiw034. <https://doi.org/10.1093/femsec/fiw034>.

Bardon, C., Poly, F., Haichar, F.E.Z., Le Roux, X., Simon, L., Meiffren, G., Comte, G., Rouifed, S., Piola, F., 2017. Biological denitrification inhibition (BDI) with procyanidins induces modification of root traits, growth and N status in *Fallopia x bohemica*. *Soil Biol. Biochem.* 107, 41–49. <https://doi.org/10.1016/j.soilbio.2016.12.009>.

Barney, J.N., Tharayil, N., DiTommaso, A., Bhowmik, P.C., 2006. The biology of invasive alien plants in Canada. 5. *Polygonum cuspidatum* Sieb. & Zucc. [= *Fallopia japonica* (Houtt.) Ronse Decr.]. *Can. J. Plant Sci.* 86, 887–905. <https://doi.org/10.4141/P05-170>.

Béraud, C., Piola, F., Gervais, J., Meiffren, G., Creuzé des Châtelliers, C., Delort, A., Boisselet, C., Poussineau, S., Lacroix, E., Cantarel, A.A.M., 2024. Biological denitrification inhibition (BDI) on nine contrasting soils: an unexpected link with the initial soil denitrifying community. *Soil Biol. Biochem.* 188. <https://doi.org/10.1016/j.soilbio.2023.109188>.

Boudsocq, S., Niboyet, A., Lata, J.C., Raynaud, X., Loeuille, N., Mathieu, J., Blouin, M., Abbadié, L., Barot, S., 2012. Plant preference for ammonium versus nitrate: a neglected determinant of ecosystem functioning? *Am. Nat.* 180, 60–69. <https://doi.org/10.1086/665997>.

Brooker, R.W., Callaghan, T.V., Jonasson, S., 1999. Nitrogen uptake by rhizomes of the clonal sedge *Carex bigelowii*: a previously overlooked nutritional benefit of rhizomatous growth. *New Phytol.* 142, 35–48. <https://doi.org/10.1046/j.1469-8137.1999.00384.x>.

Canbolat, M.Y., Bilen, S., Çakmakçı, R., Şahin, F., Aydın, A., 2006. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. *Biol. Fertil. Soils* 42, 350–357. <https://doi.org/10.1007/s00374-005-0034-9>.

Cantarel, A.A.M., Pommier, T., Desclos-Theveniau, M., Diquélou, S., Dumont, M., Grassein, F., Kastl, E.-M., Grigulis, K., Laîné, P., Lavorel, S., Lemauiel-Lavenant, S., Personeni, E., Schloter, M., Poly, F., 2015. Using plant traits to explain plant–microbe relationships involved in nitrogen acquisition. *Ecology* 96, 788–799. <https://doi.org/10.1890/13-2107.1>.

Cantarel, A.A.M., Rouifed, S., Simon, L., Bourg, J., Gervais, J., Blazère, L., Poussineau, S., Creuzé Des Châtelliers, C., Piola, F., 2020. In nitrate-rich soil, *Fallopia x bohemica* modifies functioning of N-cycle compared to native monocultures. *Diversity* 12, 156. <https://doi.org/10.3390/d12040156>.

Cassini, M.H., 2020. A review of the critics of invasion biology. *Biol. Rev.* 95, 1467–1478. <https://doi.org/10.1111/brv.12624>.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., Steege, H.T., Morgan, H.D., Heijden, M.G.A.V.D., Pausas, J.G., Poorter, H., 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51, 335. <https://doi.org/10.1071/BT02124>.

Courchamp, F., Hulme, P.E., Pyšek, P., 2020. Invasion biology and uncertainty in *native range* definitions: response to Pereyra 2019. *Conserv. Biol.* 34, 1041–1043. <https://doi.org/10.1111/cobi.13528>.

Courty, P.E., Smith, P., Koegel, S., Redecker, D., Wipf, D., 2015. Inorganic nitrogen uptake and transport in beneficial plant root–microbe interactions. *Crit. Rev. Plant Sci.* 34, 4–16. <https://doi.org/10.1080/07352689.2014.897897>.

Dassonville, N., Vanderhoeven, S., Vanparys, V., Hayez, M., Gruber, W., Meerts, P., 2008. Impacts of alien invasive plants on soil nutrients are correlated with initial site conditions in NW Europe. *Oecologia* 157, 131–140. <https://doi.org/10.1007/s00442-008-1054-6>.

Dassonville, N., Guillaumaud, N., Piola, F., Meerts, P., Poly, F., 2011. Niche construction by the invasive Asian knotweeds (species complex *Fallopia*): impact on activity, abundance and community structure of denitrifiers and nitrifiers. *Biol. Invasions* 13, 1115–1133. <https://doi.org/10.1007/s10530-011-9954-5>.

Ehrenfeld, J.G., 2003. Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6, 503–523. <https://doi.org/10.1007/s10021-002-0151-3>.

Ehrenfeld, J.G., 2010. Ecosystem consequences of biological invasions. *Annu. Rev. Ecol. Evol. Syst.* 41, 59–80. <https://doi.org/10.1146/annurev-ecolsys-102209-144650>.

Elgersma, K.J., Ehrenfeld, J.G., 2011. Linear and non-linear impacts of a non-native plant invasion on soil microbial community structure and function. *Biol. Invasions* 13, 757–768. <https://doi.org/10.1007/s10530-010-9866-9>.

Gao, G.-F., Li, P.-F., Zhong, J.-X., Shen, Z.-J., Chen, J., Li, Y.-T., Isabwe, A., Zhu, X.-Y., Ding, Q.-S., Zhang, S., Gao, C.-H., Zheng, H.-L., 2019. *Spartina alterniflora* invasion alters soil bacterial communities and enhances soil N<sub>2</sub>O emissions by stimulating soil denitrification in mangrove wetland. *Sci. Total Environ.* 653, 231–240. <https://doi.org/10.1016/j.scitotenv.2018.10.277>.

Grassein, F., Lavorel, S., Till-Bottraud, I., 2014. The importance of biotic interactions and local adaptation for plant response to environmental changes: field evidence along an elevational gradient. *Glob. Chang. Biol.* 20, 1452–1460. <https://doi.org/10.1111/gcb.12445>.

Grassein, F., Lemauiel-Lavenant, S., Lavorel, S., Bahn, M., Bardgett, R.D., Desclos-Theveniau, M., Laîné, P., 2015. Relationships between functional traits and inorganic nitrogen acquisition among eight contrasting European grass species. *Ann. Bot.* 115, 107–115. <https://doi.org/10.1093/aob/mcu233>.

Guyonnet, J.P., Vautrin, F., Meiffren, G., Labois, C., Cantarel, A.A.M., Michalet, S., Comte, G., Haichar, F.E.Z., 2017. The effects of plant nutritional strategy on soil

- microbial denitrification activity through rhizosphere primary metabolites. *FEMS Microbiol. Ecol.* 93 <https://doi.org/10.1093/femsec/fix022>.
- Grime, J.P., 2001. *Plant Strategies, Vegetation Processes, and Ecosystem Properties*, 2nd Edition. John Wiley & Sons, Chichester.
- Guiasu, R.C., Tindale, C.W., 2018. Logical fallacies and invasion biology. *Biol. Philos.* 33, 34. <https://doi.org/10.1007/s10539-018-9644-0>.
- Guyonnet, J.P., Cantarel, A.A.M., Simon, L., Haichar, F.E.Z., 2018. Root exudation rate as functional trait involved in plant nutrient-use strategy classification. *Ecol. Evol.* 8, 8573–8581. <https://doi.org/10.1002/ece3.4383>.
- Hawkes, C.V., Wren, I.F., Herman, D.J., Firestone, M.K., 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecol. Lett.* 8, 976–985. <https://doi.org/10.1111/j.1461-0248.2005.00802.x>.
- Henry, S., Texier, S., Hallet, S., Bru, D., Dambreville, C., Chêneby, D., Bizouard, F., Germon, J.C., Philippot, L., 2008. Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: insight into the role of root exudates. *Environ. Microbiol.* 10, 3082–3092. <https://doi.org/10.1111/j.1462-2920.2008.01599.x>.
- Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., Van Vark, W., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Commun. Soil Sci. Plant Anal.* 31, 1299–1396. <https://doi.org/10.1080/00103620009370514>.
- Hulme, P.E., Pyšek, P., Jarošík, V., Pergl, J., Schaffner, U., Vilà, M., 2013. Bias and error in understanding plant invasion impacts. *Trends Ecol. Evol.* 28, 212–218. <https://doi.org/10.1016/j.tree.2012.10.010>.
- Ito, M., Ueki, K., Sakamoto, S., 1982. *Studies on the total vegetation control in railroad. 1. Major weeds and factors affecting their distribution. Weed Res. Jpn* 27 (1), 41–48.
- Jha, C.K., Saraf, M., 2015. Plant growth promoting rhizobacteria (PGPR): a review. *J. Agricult. Res. Dev.* 5 (2), 108–119.
- Kapitzka, K., Zimmermann, H., Martín-López, B., Von Wehrden, H., 2019. Research on the social perception of invasive species: a systematic literature review. *NeoBiota* 43, 47–68. <https://doi.org/10.3897/neobiota.43.31619>.
- Kourtev, P.S., Ehrenfeld, J.G., Häggblom, M., 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biol. Biochem.* 35, 895–905. [https://doi.org/10.1016/S0038-0717\(03\)00120-2](https://doi.org/10.1016/S0038-0717(03)00120-2).
- Koutika, L.-S., Vanderhoeven, S., Chapuis-Lardy, L., Dassonville, N., Meerts, P., 2007. Assessment of changes in soil organic matter after invasion by exotic plant species. *Biol. Fertil. Soils* 44, 331–341. <https://doi.org/10.1007/s00374-007-0210-1>.
- LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89, 371–379. <https://doi.org/10.1890/06-2057.1>.
- Legay, N., Clément, J.C., Grassein, F., Lavorel, S., Lemauviel-Lavenant, S., Personeni, E., Poly, F., Pommier, T., Robson, T.M., Mouhamadou, B., Binet, M.N., 2020a. Plant growth drives soil nitrogen cycling and N-related microbial activity through changing root traits. *Fungal Ecol.* 44, 100910 <https://doi.org/10.1016/j.funeco.2019.100910>.
- Legay, Nicolas, Grassein, F., Arnoldi, C., Segura, R., Laîné, P., Lavorel, S., Clément, J., 2020b. Studies of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake ability of subalpine plants and resource-use strategy identified by their functional traits. *Oikos* 129, 830–841. <https://doi.org/10.1111/oik.07282>.
- Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J., Li, B., 2008. Altered ecosystem carbon and nitrogen N-cycles by plant invasion: a meta-analysis. *New Phytol.* 177, 706–714. <https://doi.org/10.1111/j.1469-8137.2007.02290.x>.
- Lowe, S., Browne, M., Boudjelas, S., De Poorter, M., 2000. 100 of the World's Worst Invasive Alien Species a Selection From the Global Invasive Species Database. *Species Specialist Group (ISSG), World Conservation Union (IUCN), Auckland*.
- Luong, J.C., Villanueva, E.M., Bauman, T.A., 2023. Native and invasive bunchgrasses have different responses to trail disturbance on California coastal prairies. *Plant Ecol.* 224, 125–135. <https://doi.org/10.1007/s11258-022-01284-z>.
- MacDougall, A.S., Turkington, R., 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology* 86, 42–55. <https://doi.org/10.1890/04-0669>.
- Mack, M.C., D'Antonio, C.M., 2003. Exotic grasses alter controls over soil nitrogen dynamics in a Hawaiian woodland. *Ecol. Appl.* 13, 154–166. [https://doi.org/10.1890/1051-0761\(2003\)013\[0154:EGACSJ2.0.CO;2](https://doi.org/10.1890/1051-0761(2003)013[0154:EGACSJ2.0.CO;2).
- Maurel, N., Fujiyoshi, M., Muratet, A., Porcher, E., Motard, E., Gargominy, O., Machon, N., 2013. Biogeographic comparisons of herbivore attack, growth and impact of Japanese knotweed between Japan and France. *J. Ecol.* 101, 118–127. <https://doi.org/10.1111/1365-2745.12026>.
- McLeod, M.L., Cleveland, C.C., Lekberg, Y., Maron, J.L., Philippot, L., Bru, D., Callaway, R.M., 2016. Exotic invasive plants increase productivity, abundance of ammonia-oxidizing bacteria and nitrogen availability in intermountain grasslands. *J. Ecol.* 104, 994–1002. <https://doi.org/10.1111/1365-2745.12584>.
- Michalet, S., Rohr, J., Warshan, D., Bardou, C., Roggy, J.-C., Domenach, A.-M., Czarnes, S., Pommier, T., Combourieu, B., Guillaumaud, N., Bellvert, F., Comte, G., Poly, F., 2013. Phytochemical analysis of mature tree root exudates in situ and their role in shaping soil microbial communities in relation to tree N-acquisition strategy. *Plant Physiol. Biochem.* 72, 169–177. <https://doi.org/10.1016/j.plaphy.2013.05.003>.
- Moreau, D., Pivato, B., Bru, D., Busset, H., Deau, F., Faivre, C., Matejček, A., Strbič, F., Philippot, L., Mougel, C., 2015. Plant traits related to nitrogen uptake influence plant-microbe competition. *Ecology* 96, 2300–2310. <https://doi.org/10.1890/14-1761.1>.
- Moreau, D., Bardgett, R.D., Finlay, R.D., Jones, D.L., Philippot, L., 2019. A plant perspective on nitrogen cycling in the rhizosphere. *Funct. Ecol.* 33, 540–552. <https://doi.org/10.1111/1365-2435.13303>.
- Muranaka, T., Ishii, J., Miyawaki, S., Washitani, I., 2005. Vascular plants to be designated as invasive alien species according to the invasive alien species act of Japan. *Jpn. J. Conserv. Ecol.* 10 (1), 19–33.
- Nashiki, M., Nomoto, T., Meguro, R., Sato, K., 1986. Effect of natural conditions and management of pastures on weed invasion in cooperative livestock farms in Japan. *Weed Res. Jpn* 31 (3), 221–227.
- Osunkoya, O.O., Perrett, C., 2011. *Lantana camara* L. (Verbenaceae) invasion effects on soil physicochemical properties. *Biol. Fertil. Soils* 47, 349–355. <https://doi.org/10.1007/s00374-010-0513-5>.
- Patra, A.K., Le Roux, X., Abbadie, L., Clays-Josserand, A., Poly, F., Loiseau, P., Louault, F., 2007. Effect of microbial activity and nitrogen mineralization on free-living nitrogen fixation in permanent grassland soils. *J. Agron. Crop Sci.* 193, 153–156. <https://doi.org/10.1111/j.1439-037X.2006.00247.x>.
- Pereyra, P.J., 2020. Rethinking the native range concept. *Conserv. Biol.* 34, 373–377. <https://doi.org/10.1111/cobi.13406>.
- Pereyra, P.J., Guiasu, R.C., 2020. Debate over the importance and meaning of native range in invasion biology: reply to Courchamp et al. *Conserv. Biol.* 34, 1044–1046. <https://doi.org/10.1111/cobi.13529>.
- Prade, K., Trollenier, G., 1988. Effect of wheat roots on denitrification at varying soil air-filled porosity and organic-carbon content. *Biol. Fertil. Soils* 7. <https://doi.org/10.1007/BF00260723>.
- Pyšek, P., Jarošík, V., Hulme, P.E., Pergl, J., Hejda, M., Schaffner, U., Vilà, M., 2012. A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Glob. Chang. Biol.* 18, 1725–1737. <https://doi.org/10.1111/j.1365-2486.2011.02636.x>.
- Radersma, S., Smit, A.L., 2011. Assessing denitrification and N leaching in a field with organic amendments. *NJAS: Wageningen J. Life Sci.* 58, 21–29. <https://doi.org/10.1016/j.njas.2010.06.001>.
- Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C., Bowman, W.D., 1999. Generality of leaf trait relationships: a test across six biomes. *Ecology* 80, 1955–1969. [https://doi.org/10.1890/0012-9658\(1999\)080\[1955:GOLTRA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[1955:GOLTRA]2.0.CO;2).
- Richardson, D.M., Pyšek, P., 2006. Plant invasions: merging the concepts of species invasiveness and community invasibility. *Prog. Phys. Geogr. Earth Environ.* 30, 409–431. <https://doi.org/10.1191/030913306pp490pr>.
- Russell, J.C., Blackburn, T.M., 2017. The rise of invasive species denialism. *Trends Ecol. Evol.* 32, 3–6. <https://doi.org/10.1016/j.tree.2016.10.012>.
- Scharf, D., Eggenschwiler, H., Olde Venterink, H., Edwards, P.J., Gusewell, S., 2009. The invasive alien plant species *Solidago gigantea* alters ecosystem properties across habitats with differing fertility. *J. Veg. Sci.* 20, 1072–1085. <https://doi.org/10.1111/j.1654-1103.2009.01105.x>.
- Shimoda, M., Yamasaki, N., 2016. *Fallopia japonica* (Japanese Knotweed) in Japan: Why Is it Not a Pest for Japanese People? In *Vegetation Structure and Function at Multiple Spatial, Temporal and Conceptual Scales*. Springer International Publishing, Cham, pp. 447–473. <https://doi.org/10.1007/978-3-319-21452-8>.
- Si, C., Liu, X., Wang, C., Wang, L., Dai, Z., Qi, S., Du, D., 2013. Different degrees of plant invasion significantly affect the richness of the soil fungal community. *PLoS One* 8, e85490. <https://doi.org/10.1371/journal.pone.0085490>.
- Smerčina, D.N., Evans, S.E., Friesen, M.L., Tiemann, L.K., 2019. To fix or not to fix: controls on free-living nitrogen fixation in the rhizosphere. *Appl. Environ. Microbiol.* 85, e02546-18 <https://doi.org/10.1128/AEM.02546-18>.
- Subbarao, G.V., Rondón, M., Ito, O., Ishikawa, T., Rao, I.M., Nakahara, K., Lascano, C., Barry, W.L., 2007. Biological nitrification inhibition (BNI)—is it a widespread phenomenon? *Plant Soil* 294, 5–18. <https://doi.org/10.1007/s11104-006-9159-3>.
- Tharayil, N., Alpert, P., Bhowmik, P., Gerard, P., 2013. Phenolic inputs by invasive species could impart seasonal variations in nitrogen pools in the introduced soils: a case study with *Polygonum cuspidatum*. *Soil Biol. Biochem.* 57, 858–867. <https://doi.org/10.1016/j.soilbio.2012.09.016>.
- Thiébaud, M., Nicolas, S., Piola, F., 2020. “The fad for *Polygonum* will fade away!”: historic aspects of the propagation and success in France of the *Reynoutria* complex based on archives. *Bot. Lett.* 167 (3), 301–314. <https://doi.org/10.1080/23818107.2020.1750478>.
- Thion, C.E., Poirel, J.D., Cornulier, T., De Vries, F.T., Bardgett, R.D., Prosser, J.I., 2016. Plant nitrogen-use strategy as a driver of rhizosphere archaeal and bacterial ammonia oxidiser abundance. *FEMS Microbiol. Ecol.* 92, fiw091. <https://doi.org/10.1093/femsec/fiw091>.
- Torres, N., Herrera, I., Fajardo, L., Bustamante, R.O., 2021. Meta-analysis of the impact of plant invasions on soil microbial communities. *BMC Ecol. Evol.* 21, 172. <https://doi.org/10.1186/s12862-021-01899-2>.
- Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y., Pyšek, P., 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems: ecological impacts of invasive alien plants. *Ecol. Lett.* 14, 702–708. <https://doi.org/10.1111/j.1461-0248.2011.01628.x>.
- Warren, R.J., King, J.R., Tarsa, C., Haas, B., Henderson, J., 2017. A systematic review of context bias in invasion biology. *PLoS One* 12, e0182502. <https://doi.org/10.1371/journal.pone.0182502>.
- Xu, C.-W., Yang, M.-Z., Chen, Y.-J., Chen, L.-M., Zhang, D.-Z., Mei, L., Shi, Y.-T., Zhang, H.-B., 2012. Changes in non-symbiotic nitrogen-fixing bacteria inhabiting rhizosphere soils of an invasive plant *Ageratina adenophora*. *Appl. Soil Ecol.* 54, 32–38. <https://doi.org/10.1016/j.apsoil.2011.10.021>.
- Zhang, C.B., Liu, W.L., Luo, B., Guan, M., Wang, J., Ge, Y., Chang, J., 2020. *Spartina alterniflora* invasion impacts denitrifying community diversity and functioning in marsh soils. *Geoderma* 375, 114456. <https://doi.org/10.1016/j.geoderma.2020.114456>.

- Zhang, M., Zeng, H., Afzal, M.R., Gao, X., Li, Y., Subbarao, G.V., Zhu, Y., 2022. BNI-release mechanisms in plant root systems: current status of understanding. *Biol. Fertil. Soils* 58, 225–233. <https://doi.org/10.1007/s00374-021-01568-y>.
- Zifcakova, L., 2020. Factors affecting soil microbial processes. In: *Carbon and Nitrogen Cycling in Soil*. Springer, Singapore, pp. 439–461. [https://doi.org/10.1007/978-981-13-7264-3\\_13](https://doi.org/10.1007/978-981-13-7264-3_13).
- Zou, J., Rogers, W.E., DeWalt, S.J., Siemann, E., 2006. The effect of Chinese tallow tree (*Sapium sebiferum*) ecotype on soil–plant system carbon and nitrogen processes. *Oecologia* 150, 272–281. <https://doi.org/10.1007/s00442-006-0512-2>.