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Influence of edaphic conditions and nitrogen fertilizers on cadmium and zinc phytoextraction efficiency of *Noccaea caerulescens*

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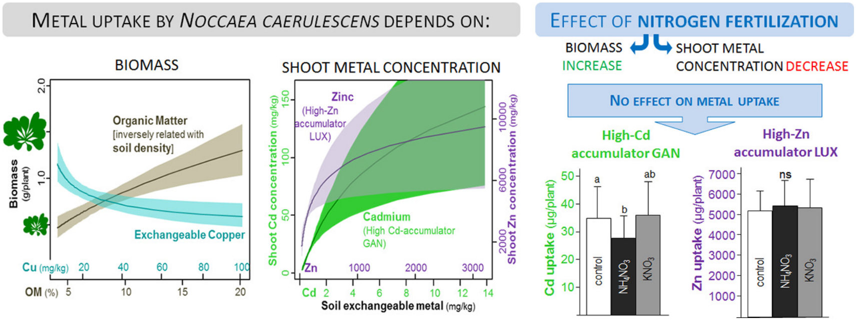
HIGHLIGHTS

- *Noccaea caerulescens* was grown on 24 soils characterized by 22 soil variables.
- Growth depended more on soil physical (organic matter) than on chemical variables.
- Low soil copper content (<50 mg kg^{-1}) and sand negatively affected shoot biomass.
- Nitrogen fertilization did not improve zinc or cadmium uptake.
- Shoot cadmium and zinc concentrations relied on soil exchangeable concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

The success of cadmium phytoextraction operations with *Noccaea caerulescens* varies by a factor of 70 between sites of trials. However, soil factors driving the efficiency of cadmium (Cd) and zinc (Zn) phytoextraction are still poorly understood, as are the effects of nitrogen fertilizers. We studied biomass production and Cd and Zn uptake by two contrasting populations of *N. caerulescens*, Ganges (metallicolous) and Wilwerwiltz (non-metallicolous) grown in pots on a range of 24 field contaminated soils for 20 weeks. The addition of KNO_3 and NH_4NO_3 fertilizers was also tested. Using model averaging of multiple regression models, we show that the major drivers of *N. caerulescens* growth are physical soil factors such as organic matter and soil bulk density while trace metal accumulation mainly relies on soil Cd and Zn exchangeable concentrations. We confirm the negative effect of soil copper (Cu) on growth, even at exchangeable concentrations below 30 mg kg^{-1}, and therefore on uptake efficiency, while increasing soil lead (Pb) content was related to increased biomass probably due to a protective effect against soil pathogens. Finally, there is a small positive effect of nitrogen fertilization on biomass production only in soils with low initial nitrogen content (under 25 µg g^{-1} NO_3^-), while above this value, the positive impact of initial nitrogen content is offset by lower shoot Cd and Zn concentrations. Our data bring substantial information regarding the physico-chemical properties to ensure *N. caerulescens* growth: a soil bulk density under 1.05 kg/dm^3, organic matter above 7% and pH under 7.5. We show that phytoextraction efficiency is maximal for moderate soil contamination in Cd (2–10 mg kg^{-1}) and Zn (300–1000 mg kg^{-1}).

1. Introduction

Noccaea caerulescens (J. Presl & C. Presl) F.K. Mey is a cadmium (Cd), zinc (Zn) and nickel (Ni) hyperaccumulator, well known to be a

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model species for the study of metal tolerance, homeostasis and hyperaccumulation (Assunção et al., 2003; Krämer, 2010). It is also considered a good candidate for Cd and/or Zn phytoextraction of soils with moderate contamination and has been tested numerous times in the field (Hammer and Keller, 2003; Jacobs et al., 2017, 2018a, b; Maxted et al., 2007; McGrath et al., 2006; Schwartz et al., 2003). In field trials large differences in biomass production of *N. caerulescens* were recorded between sites and plots within and among studies, sometimes by a factor of 5 (from 0.74 to 3.65 t ha^{-1} in McGrath et al., 2006, from 1.5 to 4 t ha^{-1} in Maxted et al., 2007 and from 1 up to 5 t ha^{-1} in Jacobs et al., 2017, 2018a). This results from differences of populations used, in cultural practices, meteorological conditions and length of growth between studies, but also to large differences in soil characteristics. Edaphic factors are also well-known to influence soil metal bioavailability (Alloway, 2012), and therefore accumulation levels in hyperaccumulator plants (Keller and Hammer, 2004; Yanai et al., 2006). This overall variability makes phytoextraction efficiency poorly predictable.

Efficiency of metal extraction depends on both shoot metal concentrations and plant biomass, the second being often neglected. Most of our knowledge on the influence of the natural variation of soil parameters on *N. caerulescens* biomass production is restricted to the influence of pH, and soil Cd and Zn to a minor extent (Maxted et al., 2007; Wang et al., 2006; Yanai et al., 2006). Yanai et al. (2006) found that at very acidic soil pH (4.4), *N. caerulescens* (*Ganges* population) showed a strong reduction of growth. In another study, Wang et al. (2006) showed that *N. caerulescens* growth was highest at the low soil pH (4.7) with high soil contamination (25.4 and 1500 mg kg^{-1}, of Cd and Zn), and at intermediate soil pH (6.1) with lower soil contamination (5 and 450 mg kg^{-1}). *Noccaea caerulescens* growth is stimulated by the presence of metals as a certain physiological need in Cd and/or Zn has been demonstrated previously for metallicolous (MET) populations, mostly *Ganges* (Escarré et al., 2000; Pongrac et al., 2009; Yanai et al., 2006), and in one case for non-metallicolous (NMET) populations (Jacobs et al., 2017). It has been shown that *N. caerulescens* has an elevated Zn deficiency threshold due to enhanced vacuolar sequestration which could explain a higher physiological need (Shen et al., 1997). It is also known that an excess in soil copper (Cu) can reduce *N. caerulescens* growth, probably due to an inhibition of iron (Fe) transport (Lombi et al., 2001b; McGrath et al., 2006; Walker and Bernal, 2004). Finally, *N. caerulescens* mostly grows on rocky shallow soils with good drainage in Belgium and Luxembourg (Dechamps et al., 2008; Meerts and Grommesch, 2001; Molitor et al., 2005) and in the Swiss Jura (Basic et al., 2006) which suggests some preference for an aerated soil structure.

Metal uptake by *N. caerulescens* has been more studied than its biomass production but most studies have so far also mostly concentrated on the soil metal load and variation in pH (Maxted et al., 2007; McGrath et al., 2001, 2006; Wang et al., 2006; Yanai et al., 2006; Zhao et al., 2003). Two studies found that Cd and Zn uptake were maximum at soil pH between 5 and 6 (Wang et al., 2006; Yanai et al., 2006). With the "Ganges" population, Yanai et al. (2006) showed that total Cd and Zn and soil pH were the best predictors of Cd and Zn concentrations and uptake on a large range of soil Cd concentrations while clay and organic carbon also explained some of the variation. Based on pot and field trials with *Ganges* plants, Maxted et al. (2007) presented a model of Cd uptake depending on Cd free ion activity in the soil solution calculated with total soil Cd, pH and soil organic carbon. Rosenfeld et al. (2018) recently highlighted the impact of soil geochemical factors (such as Fe oxide or sulfur solid phases) in addition to soil Cd and pH on *N. caerulescens* Cd uptake in four contaminated soils. Furthermore, multiple positive and negative interactions between soil trace metals of interest (Cd, Zn) and others (Cu, Fe) influence *N. caerulescens* uptake (Lombi et al., 2002; Tolrà et al., 1996; Walker and Bernal, 2004) but little is known about the overall effect on phytoextraction efficiency. There is therefore a large interest in understanding the influence of other major chemical and physical properties of the soil on *N. caerulescens* growth and to rank the relative importance of soil properties in the influence of total metal uptake, *i.e.* phytoextraction efficiency.

It is commonly accepted that one easy way to enhance aboveground biomass production of plants is through nitrogen (N) fertilization. All pot and field studies have shown a positive effect of N fertilization on *N. caerulescens* growth (Bennett et al., 1998; Schwartz et al., 2003; Sirguey et al., 2006; Monsant et al., 2008; Xie et al., 2009; Jacobs et al., 2018a). Nevertheless, N fertilization also results in lower Cd and Zn shoot concentrations due to metal dilution in leaves and/or competition with the N fertilizers in the rhizosphere, with a stronger effect with NH_4^+ than NO_3^- (Monsant et al., 2008, Xie et al., 2009, Jacobs et al., 2018a). All in all, N input increases trace metal uptake only at high doses (80–150 mg N kg^{-1} dry soil), while at lower doses of N (30 mg N kg^{-1}) it has no positive effect on metal uptake. In the field, N addition was either positive in favorable growth conditions, or neutral to slightly negative in unfavorable growth conditions (high pH and high soil density) (Jacobs et al., 2018a). We hypothesize that the effect of N fertilization will depend mostly on the initial N content and potentially on other soil fertility parameters like initial available P content.

To improve the predictability of phytoextraction, we conducted a pot trial with two contrasting populations of *N. caerulescens* for 20 weeks on 24 field soils with moderate contamination, representative of the Belgian contaminated soils where phytoextraction could be applicable. Soils were characterized by >20 physico-chemical variables and data were analyzed by means of model averaging of multiple regression models which allows for more robust inference than traditional stepward model selection. We also compared the effect of two different N treatments, NH_4NO_3 and KNO_3, as it has been suggested that *N. caerulescens* may react more strongly to NO_3^- (Schwartz et al., 2003; Sirguey et al., 2006). The objectives of the present study were 1) to investigate the effects of soil metals and physico-chemical characteristics and 2) to test the effect of moderate N fertilization, on *N. caerulescens* growth and metal uptake, assessed for two contrasting ecotypes on a wide range of field contaminated soils.

2. Material and methods

2.1. Soil sampling and analysis

Soils were collected in 24 sites (urban wastelands, nature reserves, collective and private gardens) in Brussels and Wallonia (Belgium). Sites were chosen based on a recorded trace metal contamination or based on the knowledge of a former soil use at risk according to the Belgian legislation (*e.g.* workshops for motor vehicle or metal working, printing industry, pesticide storage). Topsoil properties of the sites studied cover the range of soil pH, organic matter content (OM, %) and total trace metal concentrations reported for the French urban vegetable gardens (Joimel et al., 2016), but present systematic higher maximum values for total trace metal concentrations with factors of 20, 13, 2 and 6 for Cd, Zn, Cu, and Pb, respectively. On each site the soil was taken to 30 cm depth; 5 subsamples were brought together to form a composite. Soils were homogenized with a rotary sieve (Scheppach® RS 400) and the coarse fraction (>5 mm) was removed. General site and soil characteristics are shown in Table 1, while detailed soil physico-chemical properties can be found in Table S1.

Soil characterization was carried out following standard protocols of soil analysis (Pansu and Gautheyrou, 2006). Soil samples were air-dried and sieved through a 2 mm sieve. The remaining fraction ($2 < d < 5$ mm) was estimated as the gravel charge of the soil (GC). Soil pH-H_2O was measured using glass electrodes in a 1:5 soil:water suspension. Organic matter content was measured by loss on ignition at 500 °C. The cation exchange capacity (CEC, $cmol_c$ kg^{-1}) was measured on a soil extract with cobaltihexamine trichloride according to ISO 23470:2007. Total element concentrations were measured on a tri-acid (HCl 37%, HNO_3 65%, HF 40%) dissolution of finely ground soil samples (200 μm) on a hot

Table 1
General information on the soils used for experimentation, their locations and major chemical characteristics (other soil variables can be found in Table S1). Soils sampled are located in two regions (Brussels (B) and Wallonia (W)) and are either community gardens (CG), private gardens (PG), urban wastelands (UW) or nature reserves (NR). Values are means of analytical triplicates, and trace metal concentrations in bold are above legal thresholds in Brussels or Wallonia (for each element, the lower threshold is chosen).

Soil	Location (Region, type of site)	pH	OM (%)	N-NO_3 (mg kg^{-1})	N-NH_4 (mg kg^{-1})	Total concentrations[a] (mg kg^{-1})				Exchangeable[b] concentrations (mg kg^{-1})			
						Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
ALST	Laeken (B, CG)	6.61	20.2	187	0.72	1.4	**141**	**222**	**334**	0.70	42	70	151
AN1	Anderlecht (B, UW)	7.24	8.0	420	0.77	0.6	26	103	126	0.46	7	48	44
AN2	Anderlecht (B, UW)	7.68	4.1	0.9	0.13	1.0	49	**572**	217	0.36	14	290	40
AN3	Anderlecht (B, UW)	7.69	5.4	0.8	0.85	1.0	73	**301**	228	0.89	52	295	91
AN4	Anderlecht (B, UW)	7.85	4.5	1.0	0.48	1.2	94	**473**	226	0.35	29	195	36
ANG	Angleur (W, PG)	6.82	13.6	5.4	9.24	**3.7**	107	**719**	**1700**	3.01	38	209	730
BEM	Forest (B, CG)	7.13	6.0	66	1.63	1.2	49	182	227	0.91	18	72	104
CHA1	Châtelet (W, PG)	7.00	16.6	7.5	1.75	1.7	86	**247**	**592**	0.76	19	66	177
CHA2	Châtelet (W, PG)	7.24	19.9	2.0	0.67	1.2	84	**419**	**479**	0.58	13	103	164
FLO	Flône (W, PG)	6.96	9.4	13.5	28.6	**6.2**	32	198	**1460**	5.41	10	121	730
GR	Laeken (B, UW)	6.76	19.9	858	1.07	1.3	94	**279**	**315**	0.87	33	113	127
GRAY	Ixelles (B, CG)	7.63	5.6	27.4	0.76	1.3	**172**	**262**	**2390**	0.61	80	120	830
LIE	Liège (W, PG)	6.24	16.9	6.2	28.9	**20.0**	**240**	**841**	**5320**	13.86	101	383	3200
MAS	Brussels (B, UW)	8.01	5.0	39.1	0.52	1.6	**205**	**287**	**427**	0.67	39	94	51
MOE1	Evere (B, NR)	6.84	10.5	174	0.43	**2.0**	70	**1480**	**2650**	1.37	24	580	850
MOE2	Evere (B, NR)	7.18	8.7	750	2.29	1.0	26	**1410**	209	0.37	7	710	75
NAV	Schaerbeek (B, CG)	7.47	7.0	3.2	0.40	1.3	110	**263**	278	0.50	46	102	76
RUE	Molenbeek (B, CG)	7.93	4.3	0.8	0.38	1.1	28	152	188	0.17	7	47	30
SER1	Seraing (W, PG)	7.20	13.5	2.1	1.03	**2.8**	88	**412**	**735**	1.52	23	114	232
SER2	Seraing (W, PG)	6.95	13.3	19.7	1.33	**2.6**	69	181	**597**	1.64	16	72	235
SOL6	Vaux-sous-Chèvremont (W, PG)	7.35	9.8	1.3	0.93	**3.0**	53	154	**584**	1.91	16	59	180
TIL	Uccle (B, CG)	6.26	4.4	83	1.44	0.6	29	92	75	0.29	17	68	48
TIN	Tinlot (W, PG)	7.01	7.5	1.2	4.12	1.6	40	**525**	**593**	0.82	13	175	247
WIE	Forest (B, CG)	7.82	8.1	0.9	0.46	0.9	84	**301**	**307**	0.21	18	56	51
	MIN	6.24	4.1	0.8	0.13	0.6	26	92	75	0.17	7	47	30
	MAX	8.01	20.2	858	28.9	20	240	1480	5320	13.86	101	710	3200
Legal intervention thresholds for agricultural soils[c]					**Brussels**	**2**	**120**	**200**	**333**				
					Wallonia	**10**	**145**	**400**	**300**				

[a] Tri-acid dissolution.

[b] EDTA-NH_4^+ acetate extraction.

[c] Based on aqua regia dissolution.

plate at 70 °C for 48 h. The dry residue was re-dissolved with 1 mL HNO_3 65% in a volume of 30 mL and total element concentrations were determined by inductively coupled plasma emission spectrometry (ICP-OES) (Varian Vista-MPX) and checked with a certified reference soil material (SRM 2711a, Montana II Soil). Exchangeable cations (Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Ni, Pb, Zn) were extracted with ammonium acetate 0.5 M-EDTA 0.02 M at pH 7 (Cottenie et al., 1979) and their concentrations were measured by ICP-OES (Varian Vista-MPX). Soil texture (percentage of clay, silt and sand) was determined using wet sieving and the pipette method after OM destruction with H_2O_2 and Na citrate dispersion of clay. Undisturbed soil cores were taken after cultivation for soil bulk density (BD) measurement (cylinder method). A water permeability index (PI) was measured as the infiltration speed of 50 mL water over 6 h in undisturbed soil cores previously watered to water-holding capacity. A carbonate index (CARB) was determined by a simple reaction to 10% HCl with a binary response: 0 (no or very weak reaction) or 1 (strong visual reaction). Mineral NO_3^- and NH_4^+ exchangeable concentrations were measured on 1 M KCl extract of fresh soil samples collected in pots before the first N application by the sulphanilamide colorimetric method for NO_3^- and indophenol blue method for NH_4^+, and were expressed in units of N (N-NO_3 and N-NH_4 in mg kg^{-1}).

2.2. Pot experiment and plant analysis

Plastic pots of 500 cm^3 were filled with 400 g of air-dried soil mixed with 150 g of quartz gravel (6–12 mm) to maintain aeration and limit compaction. Our aim in adding this gravel fraction was to avoid artificial compaction of soils that had previously been sieved. The presence of this coarse material did not affect the soil variables measured on the fine fraction (<2 mm). Plants were grown from seeds for two months in trays filled with commercial garden compost placed on a sheltered terrace. Seedlings were transplanted in early April 2017 at the four-to-six leaves stage in 516 pots with contaminated soils (24 soils × 2 populations × 3 treatments × 3–4 replicates). To avoid any fertilizer or metal leaching, we put all replicates of the same soil and fertilizer treatment in the same watering tray. To avoid local environmental effect in the unheated plastic greenhouse, we randomized watering trays every two weeks. Two populations of *Noccaea caerulescens*, one metallicolous (MET) from Ganges, hereafter GAN (village of Saint-Laurent-le-Minier, South of France), and one non-metallicolous (NMET) from Luxembourg, hereafter LUX (village of Wilwerwiltz) were used. After two weeks, dead plants were replaced (post-transplantation mortality). Nitrogen treatment (NH_4NO_3 or KNO_3, in solution) was added for the first time one month after transplantation and then every three weeks (each time 10 mg N kg^{-1} dry soil (DS) for a total of 40 mg N kg^{-1} DS) while no N was added to control pots. Plants were watered every week with distilled water. Pychlorex (chlorpyriphos 5%) was applied once in all pots following an invasion of sciarid flies larvae. All flower stalks were cut off the first week of June and August to promote vegetative growth.

After 20 weeks of growth aboveground plant parts were harvested, including leaves (*i.e.* vegetative biomass) and flower stalks (*i.e.* reproductive biomass). Mortality was estimated as the number of dead plants at the end of the experiment. Plants were thoroughly washed with tap water and rinsed with deionized water. Reproductive and vegetative biomass of 65 °C oven-dried plants was measured. For each plant, reproductive and vegetative parts were ground together with a Retsch ZM100 mill and subsamples of 0.4 g were dry-ashed for 12 h at 450 °C and dissolved with concentrated HNO_3 (65%). Plant concentrations in trace metals (Cd, Cu, Ni, Pb, Zn) macro- and micronutrients (calcium: Ca, magnesium: Mg, potassium: K, phosphorus: P, iron: Fe, and manganese: Mn) – expressed as μg g^{-1} of 105 °C-dried plant – were measured by ICP-OES (Varian, Vista MPX). Method accuracy was checked with NIST standard reference (SRM 1547, Peach leaves) and an in-house standard. Plant metal uptake (expressed in µg plant^{-1}) was calculated as the product of plant biomass and shoot Cd or Zn concentrations.

2.3. Statistical analysis

Plant and soil variables were transformed using the Box-Cox function prior to all analyses to linearize relations among explanatory and dependent variables. The variations in biomass production (vegetative and reproductive) and Cd and Zn concentrations and uptake were analyzed by means of three-way analysis of variance to investigate the effects of soil, N treatments (NH_4NO_3 or KNO_3) and population (GAN and LUX). We conducted analyses of covariance of biomass production to test the interaction between initial soil N content (N-NH_4 and N-NO_3 as covariables) and the treatment effect. Data transformation and analysis of variance were performed using the package 'car' in R (Fox et al., 2011).

To determine which soil factors influence the most biomass production, shoot metal concentrations and metal uptake, we used a model averaging approach (Burnham and Anderson, 2002) to construct a linear regression model based on data obtained on the control treatment. In this model, we used as predictor variables exchangeable concentrations in trace metals and macronutrients instead of total concentrations because: 1) the former are more representative of short term availability and informative for plant-soil interactions, and 2) metal uptake by *N. caerulescens* is better explained by labile or exchangeable trace metal concentrations (Hutchinson et al., 2000; Knight et al., 1997; Sterckeman et al., 2004). The CARB variable was considered as a quantitative discontinuous variable. Such binary descriptor can be used in principal component analysis or regression analysis models (Legendre and Legendre, 2012). First, we conducted a selection among the 22 soil variables measured to reduce collinearity to a maximum correlation of 0.7 between two variables using the *vifcor* function in package *usdm* (Naimi et al., 2014). One exception was made for soil Cd and Zn exchangeable concentrations which were both kept for further analysis despite their correlation ($r = 0.85$). We scaled and centred the 16 remaining selected soil variables to allow for comparison between variable estimates. All possible linear models were constructed using a combination of the selected soil variables as explanatory variables (with a maximum of 10 variables in each model), to account for biomass production, shoot Cd and Zn concentrations, and Cd and Zn uptake. The models were first ranked according to their corrected Akaike Information Criterion (AICc). Model averaging was performed with the function *model.select* (for similar use, see Simon-Delso et al., 2017) using shrinkage averaging over all possible models to calculate AICc weights of importance, average coefficients and unconditional standard errors for each variable (Burnham and Anderson, 2002). This method shrinks the average coefficients towards 0 for variables mostly present in the poorly supported models. The AICc variable weights (*i.e.* the probability for a given variable to be present in the best model if data is resampled) enable the comparison of relative importance of variables. A reasonable minimum threshold of 0.7 of AICc variable weight was chosen as a criterion to interpret only the most important variables. Results were confirmed by a second method from the *glmulti* package which conducts model averaging over the 100 best models (*i.e.* with the lowest AICc) with an iterative algorithm (Calcagno and de Mazancourt, 2010). All statistical analyses were conducted with the R statistical software v 3.4.4 (R Development Core Team, 2018).

3. Results

3.1. Growth and metal accumulation on 24 unfertilized soils: differences between populations

The average survival rate on the 24 control soils was 89%, most of the mortality being probably due to pests (sciarid flies larvae, *personal observation*). Average biomass production, shoot macronutrients and trace metal concentrations on the 24 control soils can be found in Table S2. After 20 weeks, average aboveground plant biomass on the control soil (without N fertilization) was 1.08 g (LUX 1.12 g and GAN 1.04 g, $p > 0.05$) and ranged across soils from 0.21 g (RUE) to 3.3 g (GR) (Fig. 1). GAN plants produced on average more reproductive biomass

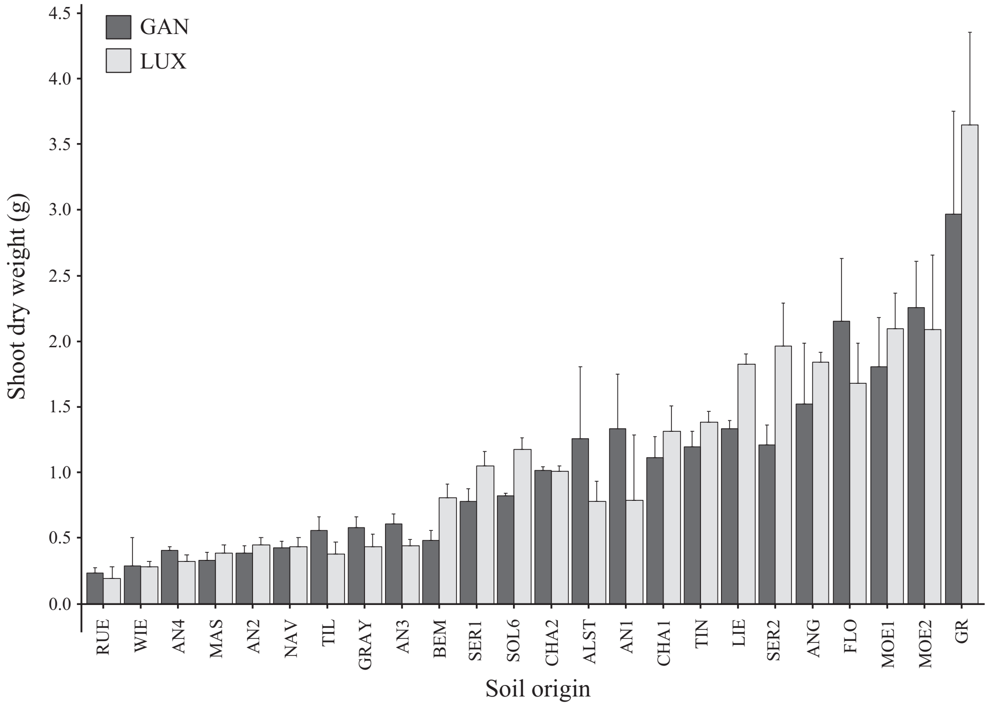


Fig. 1. Shoot biomass (vegetative plus reproductive) of *N. caerulescens* plants from Ganges (GAN) and Luxembourg (LUX) populations grown on 24 different soils for 20 weeks (control treatment). Data are means + standard errors ($n = 2$–4 plants per population × soil), ranked by increasing mean values of both populations.

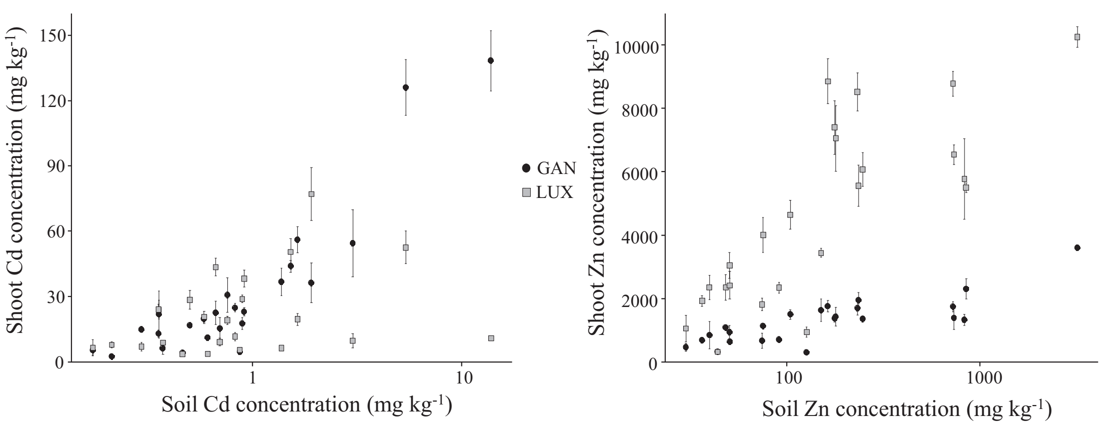


Fig. 2. Shoot Cd and Zn concentrations in *N. caerulescens* plants (Ganges, GAN; Luxembourg, LUX, populations) grown on 24 different soils for 20 weeks (control treatment), as a function of soil Cd and Zn exchangeable concentrations (EDTA-NH_4^+ acetate) (logarithmic scale). Data are means ± standard errors ($n = 2$–4 plants per population × soil).

(flower stalks) (0.37 g) than LUX (0.13 g) ($p < 0.001$), whereas vegetative biomass of GAN plants (0.68 g) was lower than LUX (0.99 g) ($p < 0.001$) (Table S3).

As expected, shoot Cd concentrations were overall higher in GAN plants (31 μg g^{-1}, ranging from 3 to 138 μg g^{-1}) than in LUX plants (22 μg g^{-1}, ranging from 4 to 77 μg g^{-1}) ($p < 0.001$), the difference being more pronounced on soils with higher contamination (Fig. 2). Conversely shoot Zn concentrations were significantly higher in LUX plants (4290 μg g^{-1}, from 320 to 10,250 μg g^{-1}) than in GAN plants (1250 μg g^{-1}, from 300 to 3600 μg g^{-1}) on all soils ($p < 0.001$) (Fig. 2).

Correlations between macronutrient and trace metal concentrations in shoots were calculated at the species and population levels on the control (only significant correlations with $|r| > 0.3$ are indicated). For shoot macronutrients there was a significant correlation between Ca and Mg ($r = 0.36$) and K and P ($r = 0.40$) at the species level. For trace metals, Cd, Zn and Ni were correlated (Cd-Zn: $r = 0.40$, Cd-Ni: $r = 0.45$, Zn-Ni: $r = 0.43$). Correlations were stronger and not always of the same nature when considering populations separately: for GAN, Ca and Mg ($r = 0.55$), K and P ($r = 0.65$), Cd and Zn ($r = 0.80$), Cd and Mn ($r = 0.50$) and Ni and Cu ($r = 0.44$); for LUX, K and P ($r = 0.50$), Cd and Zn ($r = 0.51$), Cd and Ni ($r = 0.74$), Ni and Cu ($r = 0.48$), Zn and Mn ($r = 0.56$).

3.2. Influence of soil parameters

The influence of soil parameters on biomass production and metal accumulation was assessed by means of a selection of linear models with model averaging performed on the two populations separately. A principal component analysis was performed to synthesize the explanatory dataset (Fig. S4). Three groups of collinear soil variables (correlation $r > 0.7$) were identified in the dataset, and only one variable per group was kept as explanatory variable for model selection and chosen to minimize collinearity with other variables: **OM** which was correlated with CEC and BD was kept, **Sand** with Silt and Clay, **Fe** with pH and Al. A threshold of AICc variable weight of importance $w > 0.7$ was set to identify the most important explanatory variables significantly supported by the models; for instance, a factor with a $w = 0.95$ was kept in 95% of the 100 best regression models.

For biomass production, selected soil explanatory variables were the same for both populations. Coefficient estimates (in parentheses) indicate the magnitude and the sign of the effect on the response variable. Organic matter content (GAN 0.3, LUX 0.38), Pb (GAN 0.32, LUX 0.36) and initial N-NO_3 (GAN 0.3, LUX 0.24) positively influenced biomass production while Cu (GAN -0.18, LUX -0.12) and Sand (GAN -0.19, LUX -0.18) had a negative influence (Fig. 3). In order to rule out

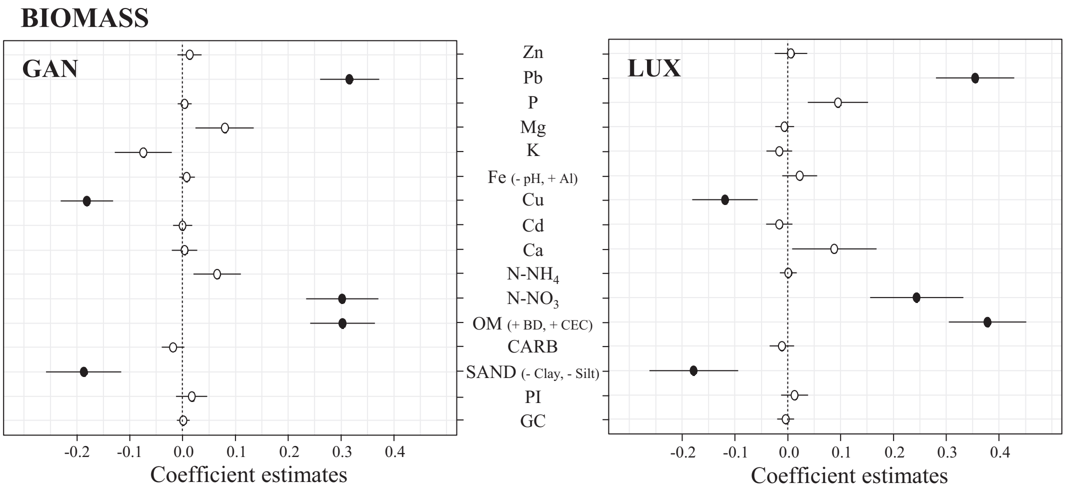


Fig. 3. Results of model averaging of linear regression models aiming at explaining the variation of biomass production of *N. caerulescens* as a function of 16 soil variables (lines) in two populations (GAN: Ganges; LUX: Luxembourg). Coefficient estimates are the averaged coefficients for each standardized soil variable (± unconditional standard error), solid circles for the variables significantly supported by the models (AICc weight $w > 0.7$), empty circles for the variables with AICc weight $w < 0.7$. Variables between brackets are positively (+) or negatively (−) correlated ($|r| > 0.7$) to the variable retained in model selection. CARB = Carbonate index, PI = Permeability index, GC = Gravel charge.

potential effects of outliers and interpret with confidence the effect of Pb, we performed new model selection without three soils with extreme Pb and Zn values (LIE, MOE1 and MOE2) and the results were mostly similar (Table S4). Effect curves of the selected variables enable to show that the effect of some variables occurred mostly in a specific range – between 0 and 50 mg kg^{-1} for N-NO$_3$, 0 and 300 mg Pb kg^{-1} and 0 and 30 mg Cu kg^{-1} – while for OM and Sand the effect was almost linear (Fig. S1).

Shoot Cd concentrations were mainly explained by soil Cd for both populations (GAN 1.19, LUX 1.81) (Fig. 4). A negative influence of initial N-NO$_3$ on shoot Cd concentrations was highlighted for both populations (GAN -0.32, LUX -0.45). In LUX plants, shoot Cd concentration was also negatively influenced by soil Zn (−0.98), and to a minor extent by gravel charge (GC; −0.36) and Pb (−0.26). In GAN plants the CARB index (−0.25) and Ca (−0.23) had a small negative influence on Cd accumulation.

Shoot Zn concentration was also mainly influenced by soil Zn (GAN 0.43, LUX 0.75) but also by soil Cd (GAN 0.36, LUX 0.61) (Fig. 5). In LUX plants, shoot Zn concentration was also negatively influenced by N-NO$_3$ (−0.62) and GC (−0.31), and positively by Sand (0.54), while GAN plants were negatively influenced by NH$_4$ (−0.17) and Ca (−0.28).

On the whole, Cd and Zn uptake (*i.e.* product of biomass and shoot Cd or Zn concentrations) were explained by a combination of the above mentioned variables, with however trade-off for variables who had opposed effects on the two components of the uptake. Cadmium uptake mostly depended on soil Cd (1.94) and Zn (−0.91), and secondarily OM (0.41), Cu (−0.32) and GC (−0.31) for LUX, while for GAN population soil Cd (1.21), CARB (−0.51), Cu (−0.41) and OM (0.3) were the main explanatory variables (Fig. 4). Zinc uptake depended mostly on soil Cd (or Zn) (0.91) and OM (0.92) but also P (0.5) and Pb (0.38) for LUX, and on Zn (0.71), OM (0.49) and Cu (−0.31) for GAN (Fig. 5). Effect curves of the selected variables on Cd uptake showed that soil Cd was by far the most influential variable and that the negative effect of soil Zn on LUX uptake occurred mostly between 0 and 300 mg Zn kg^{-1} (Fig. S2). For Zn uptake, the effect curves highlighted the larger influence of soil determinants other than soil Zn compared to Cd uptake (Fig. S3).

3.3. Effect of nitrogen fertilization

The N treatments alone did not consistently increase biomass production over all soils, but the interaction between treatment and soil factor was highly significant ($p < 0.001$) (Table S3), suggesting that N input had different effects on biomass depending on soil properties. To elucidate the soil × treatment interaction, we performed an analysis of covariance using both initial soil concentrations in NH$_4^+$ and NO$_3^-$ as covariables, with both populations pooled since there was no population effect on biomass production. Both NH$_4^+$ and NO$_3^-$ were significant covariables of the biomass production ($p < 0.001$ and $p < 0.01$, respectively) but only NO$_3^-$ had a significant interaction with the treatment effect ($p < 0.01$)

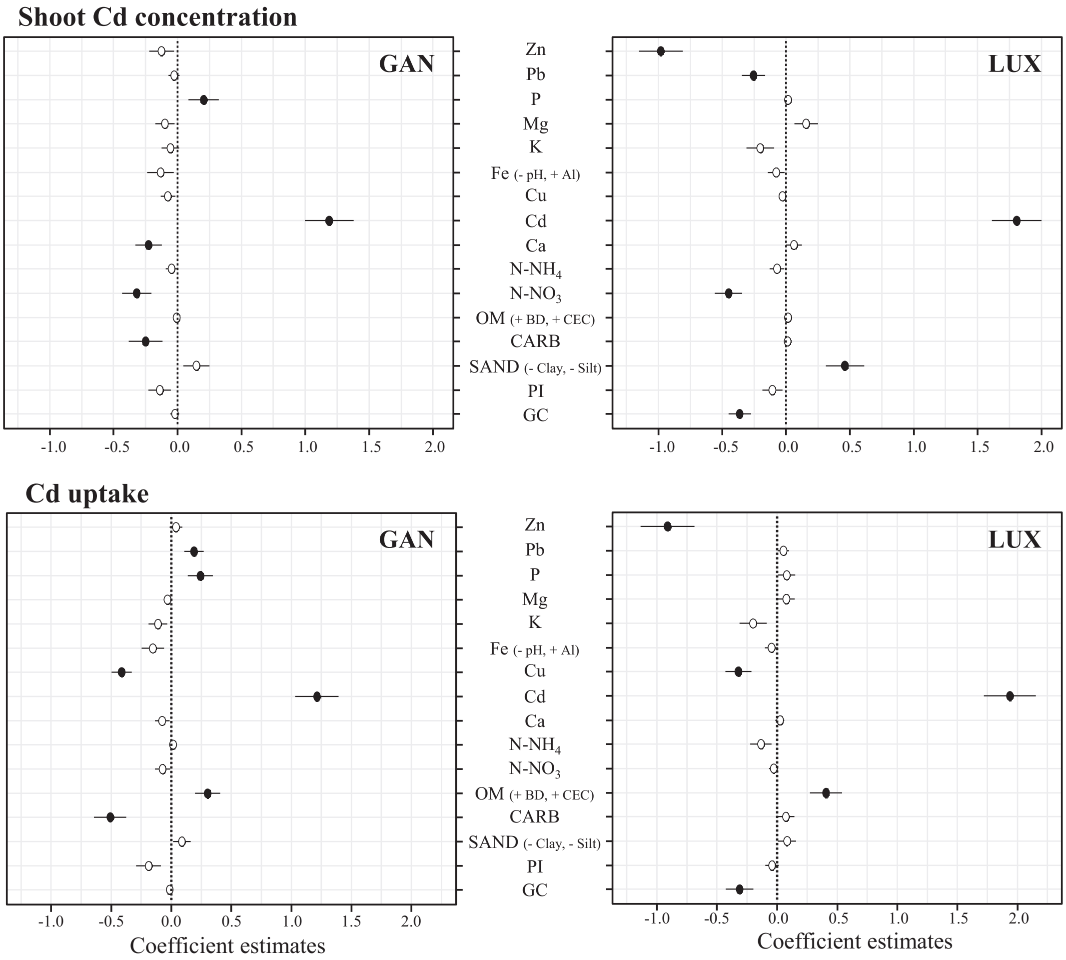
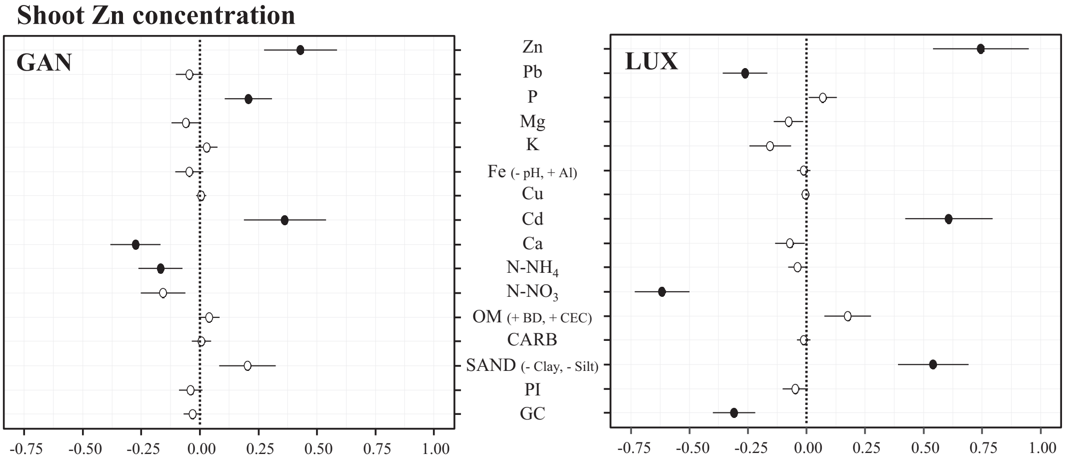
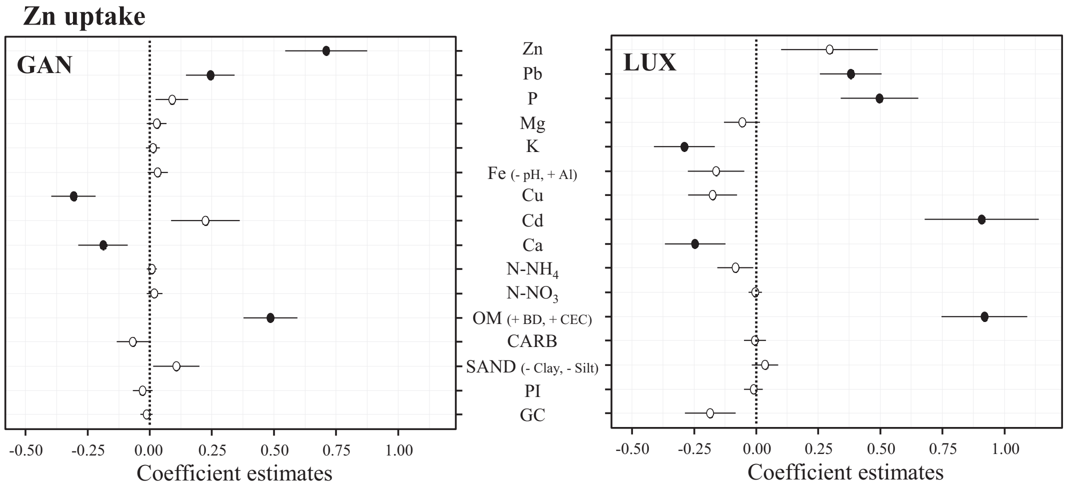


Fig. 4. Results of model averaging of linear regression models aiming at explaining the variation of shoot Cd concentration and Cd uptake of *N. caerulescens* as a function of 16 soil variables (lines) in two populations (GAN: Ganges; LUX: Luxembourg). Coefficient estimates are the averaged coefficients for each standardized soil variable (± unconditional standard error), solid circles for the variables significantly supported by the models (AICc weight w > 0.7), empty circles for the variables with AICc weight w < 0.7. Variables between brackets are positively (+) or negatively (−) correlated (|r| > 0.7) to the variable retained in model selection. CARB = Carbonate index, PI = Permeability index, GC = Gravel charge.



Shoot Zn concentration



Zn uptake

Fig. 5. Results of model averaging of linear regression models aiming at explaining the variation of shoot Zn concentration and Zn uptake of *N. caerulescens* as a function of 16 soil variables (lines) in two populations (GAN: Ganges; LUX: Luxembourg). Coefficient estimates are the averaged coefficients for each standardized soil variable (± unconditional standard error), solid circles for the variables significantly supported by the models (AICc weight w > 0.7), empty circles for the variables with AICc weight w < 0.7. Variables between brackets are positively (+) or negatively (−) correlated (|r| > 0.7) to the variable retained in model selection. CARB = Carbonate index, PI = Permeability index, GC = Gravel charge.

(Table S5). This indicates that there was no positive effect of the treatment (but rather a negative one) on biomass production at the highest initial soil NO_3^- content (Fig. 6). We then performed a second analysis of variance of biomass production including only the soils with N content under 25 mg N-NO_3 kg^{-1} DS (17 out of 24) because it corresponds approximately to the threshold value (Fig. 6), and the treatment effect was then significant ($F_{2,234} = 4.83$, $p < 0.01$, where $F_{x,y}$ is the F value of the treatment factor, x and y are the degrees of freedom of the treatment and the residuals, respectively). Considering only those soils, the average total biomass was higher on NH_4NO_3 and KNO_3 treatments compared to the control, by 15% for GAN and 22% for LUX.

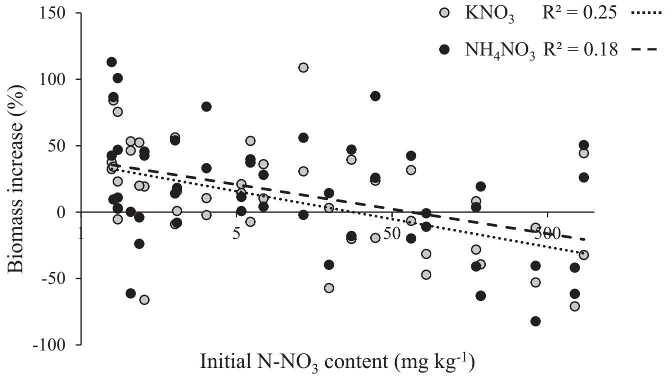


Fig. 6. Effect of two nitrogen treatments (KNO_3 and NH_4NO_3) on *N. caerulescens* biomass (Ganges, GAN, and Luxembourg, LUX populations pooled) depending on the initial N-NO_3 content in the 24 different soils (logarithmic scale). Treatment effect is calculated as (MB_T - MB_C)/MB_C where MB is the mean biomass for one population on one soil either on one of the two treatments (MB_T) or on the control (MB_C).

N addition significantly influenced both Cd ($p < 0.001$) and Zn ($p < 0.001$) shoot concentrations, but differently depending on the nitrate salt. Ammonium nitrate addition had a negative effect on metal concentrations (Fig. 7). Shoot Cd concentration was higher in plants of both populations grown on the control (26 μg g^{-1}) and on the KNO_3 treatment (24 μg g^{-1}) than on the NH_4NO_3 treatment (20 μg g^{-1}) (Table S3). Shoot Zn concentrations for the LUX population were lower on NH_4NO_3 (4190 μg g^{-1}) and KNO_3 (4260 μg g^{-1}) treatment than on the control (4630 μg g^{-1}) while there was no effect for GAN population (Table S3). Overall, the treatment effect on Cd uptake (product of biomass and shoot Cd concentration) was significant, as Cd uptake was about 15% lower with the NH_4NO_3 treatment compared to the control ($p < 0.001$) while there was no significant effect of the KNO_3 treatment but there was however a significant interaction between soil and treatment. The treatment had no pure effect on Zn uptake, but once again soil × treatment interaction was significant. When considering only the 15 soils with <25 mg N-NO_3 kg^{-1} DS, there was no significant treatment effect on Cd and Zn uptake which

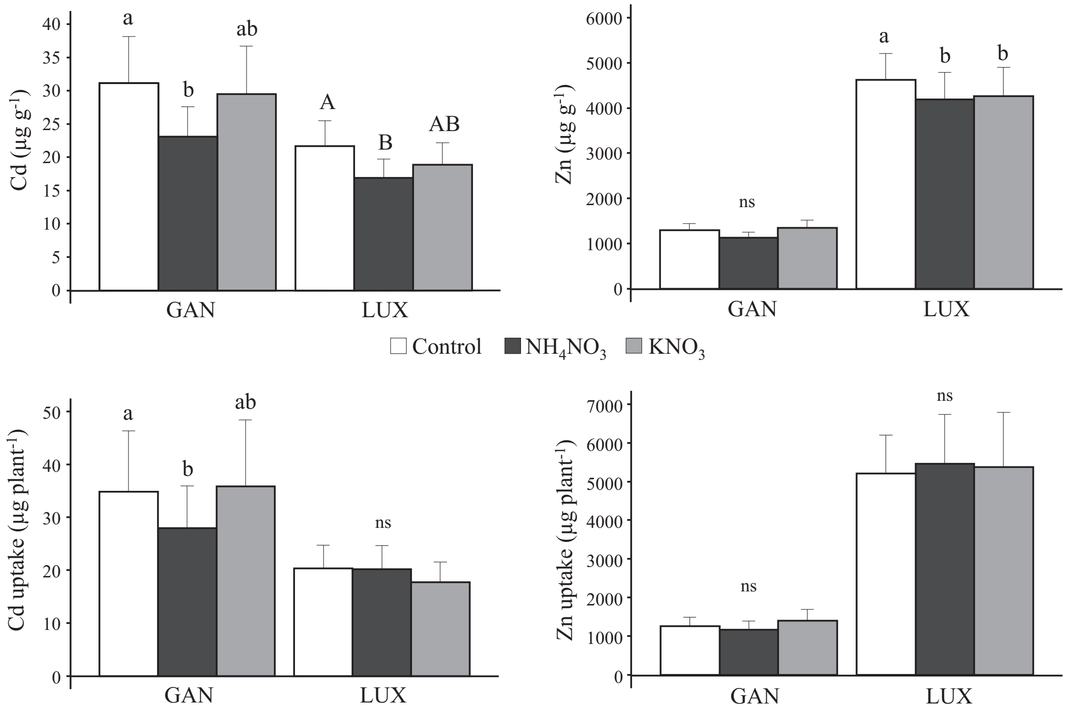


Fig. 7. Effect of the two nitrogen treatments (KNO_3 and NH_4NO_3) on Cd and Zn concentrations and uptake of *N. caerulescens* plants (Ganges, GAN, and Luxembourg, LUX populations) across 24 soils. Data are means + standard errors ($n = 68–80$ plants). Different letters indicate significant differences among nitrogen treatments for each population separately.

shows that the biomass gain was offset by reduced shoot Cd and Zn concentrations.

3.4. Metal removal

Average metal uptake was calculated as the proportion of the metal mass initially present in the pot that was harvested in the aboveground biomass (metal mass in the plant/metal mass in the soil). Mean Cd uptake across all soils and treatments is about 5% of the exchangeable Cd content (ranging from 2 to 10%) and 3% of the total Cd content (ranging from 0.3% to 6%) with GAN population (Fig. 8). Cadmium uptake with LUX population is not significantly different from GAN except on highly contaminated soils, where it is smaller. On the contrary, LUX population yielded a higher Zn uptake, on average 6% of the exchangeable content (ranging from 1 to 14%) and 2% of the total content (ranging from 0.3% to 4.4%) compared to 2% of the exchangeable content (ranging from 0.2 to 4%) and 0.6% of the total content (ranging from 0.1% to 1.6%) with GAN population (Fig. 8). Cd extraction efficiency with GAN increases with soil contamination, being maximal for total concentrations between 2 and 10 mg Cd kg^{-1} (7.6% and 5.5% of exchangeable and total content), while on the only soil with higher contamination (LIE 20 mg Cd kg^{-1}) the extraction rate was much lower (2 and 1.4%). Zn extraction with LUX is also more efficient at intermediate contamination level (300 and 1000 mg Zn kg^{-1}) than at low (<300 mg Zn kg^{-1}) or very high (1000 mg Zn kg^{-1}) levels of contamination.

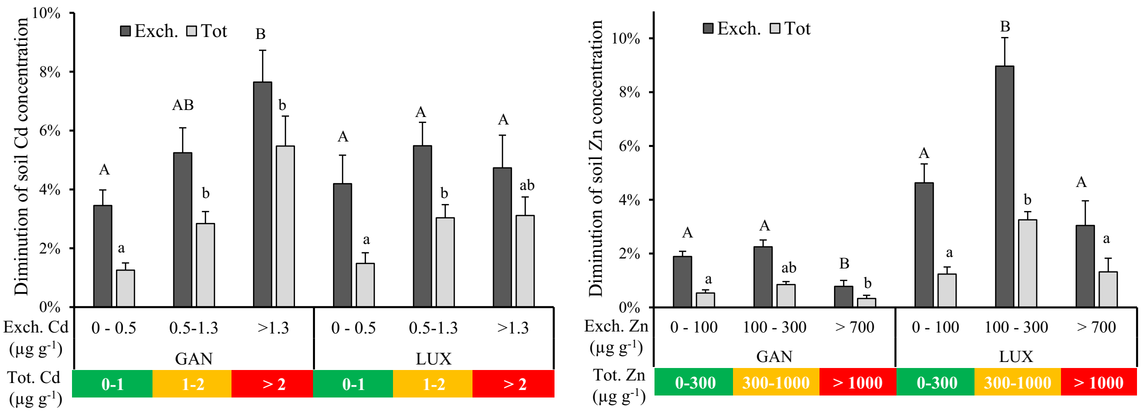


Fig. 8. Average Cd and Zn removal by *N. caerulescens* on all treatments pooled, expressed as the diminution of exchangeable (EDTA-NH_4^+ acetate extraction) and total Cd and Zn concentrations in pots, on 23 soils (LIE soil was here removed because of its extreme Cd and Zn concentrations). For each trace metal (Cd and Zn), soils were grouped in three classes according to their exchangeable concentrations in Cd and Zn (which more-or-less correspond to the total concentrations indicated, based on the Belgian norms for trace metal in agricultural soils): no or very low contamination (green, $n = 7$ (Cd) or 10 (Zn)), moderately low contamination (orange $n = 10$ (Cd) or 9 (Zn)), moderate to high contamination (red $n = 6$ (Cd) or 4 (Zn)). GAN: Ganges population; LUX: Luxembourg population. For each population, different letters indicate significant difference between classes of soil contamination at the $p < 0.05$ level (*t*-tests with Šidák correction). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

In this study we have compared for the first time two contrasting populations of *N. caerulescens* on a wide range of moderately contaminated soils to assess the influence of natural soil variation on phytoextraction efficiency. We also investigated the effect of a common agricultural practice, N fertilization, on phytoextraction. The originality of this work was to study the effect of >16 soil variables on both biomass and metal concentrations – the former often being neglected when considering extraction efficiency – with a realistic growth length (20 weeks).

4.1. Influence of soil factors on biomass production

Plant biomass production varied by more than tenfold among soils, which indicates a strong influence of soil properties. Sterckeman and Puschenreiter (2018) pointed out that Cd phytoextraction efficiency varies by a factor of 70 between sites.

Interestingly, GAN and LUX populations showed similar growth responses to soil variables. First, *N. caerulescens* growth was clearly stimulated by higher organic matter (OM) content and lower bulk density (BD). This positive influence could be interpreted by the benefits of soil OM on chemical and physical properties: higher fertility and a greater soil macroporosity. Nevertheless, since none of the major cations (K, P, and Mg) and N forms are correlated with OM ($r < 0.5$, Table S6), the effect on chemical fertility seems less important. This rather supports the field observation of a high sensitivity of the species to soil compaction, and that natural populations mostly grow on shallow and coarse substrates (Basic et al., 2006; Molitor et al., 2005; Sirguey et al., 2018). Our results suggest that *N. caerulescens* grows best in soils with OM > 7% and soil bulk density < 1.05 kg/dm^3. In a pot experiment on 7 soils, Knight et al. (1997) obtained data showing a positive correlation between soil organic carbon and *N. caerulescens* biomass production ($r = 0.9$ using Tables 1 and 2 data from Knight et al., 1997). In a field trial on 10 soils, Maxted et al. (2007) found no significant correlation between OM and biomass but the best yield was still obtained on a soil with high OM content (22%) while the lowest was on a clayey soil with lower OM (5–10%). In this context, the negative influence of sand content (varying from 30 to 80%) in the same model (and hence positive of clay and silt) on growth may seem counterintuitive, but it could be interpreted as a positive effect of increasing clay and silt content for water retention in these relatively sandy soils. *Noccaea caerulescens* could have a preference for soils with good drainage but with a minimum water retention capacity to prevent excessive drying.

Another variable that positively influences biomass is N as available nitrate ($N\text{-}NO_3$). The dominant N form in most soils was NO_3^- which could explain why NO_3^- rather than NH_4^+ was selected as a predictor of biomass production. We here confirm the positive impact of increasing N on *N. caerulescens* growth on a large range of field soil concentrations in $N\text{-}NO_3$ (from 1 to 858 mg kg^{-1} DS, median 7 mg kg^{-1} DS) which had already been demonstrated earlier with synthetic fertilizers (Schwartz et al., 2003; Xie et al., 2009).

Our models also highlighted that two trace metals, Cu and Pb, influence biomass production, positively for Pb and negatively for Cu. Cu concentrations in the four most contaminated soil range from 140 and 240 mg kg^{-1}, and appear to inhibit *N. caerulescens* growth. Such growth inhibition had already been demonstrated in previous works but at higher concentrations (1200 mg kg^{-1}) (Lombi et al., 2001b; Walker and Bernal, 2004). The positive influence of Pb on both populations is more unexpected and less straightforward to explain. Growth stimulation of *N. caerulescens* by trace metals has already been shown for Cd and Zn (Escarré et al., 2000; Pongrac et al., 2009) – the two hyperaccumulated trace metals – but never in the case of Pb. We know that *N. caerulescens* can grow on sites with very high Pb contamination (frequently between 10,000 and 20,000 mg kg^{-1}; Escarré et al., 2011) and therefore it makes sense that no negative effect of Pb is found here. A potential covariation with other trace metals (Cd, Cu and Zn) or OM can be ruled out since Pb is not strongly correlated ($r < 0.5$) to any other soil variable. Furthermore, models without Pb explain less variation in biomass and had a higher AICc value. The most convincing explanation would be a direct effect of Pb on soil pathogens and pests, which could therefore lead to an indirect effect on plant growth by decreasing the negative impact of plant root feeders and pathogens. The main pest and pathogens of *N. caerulescens* previously identified are sciarid flies larvae (in controlled conditions, personal observation), and fungal diseases causing damping off and root rot (*Phytophthora* sp., *Fusarium* sp. or *Pythium* sp.) (Maxted et al., 2007; Simmons et al., 2015). The inhibition of soil pathogen development by elevated soil Pb content has already been demonstrated (Harris and Birch, 1988; Ngu et al., 1998). This hypothesis is also supported by the significant correlation between survival rate and soil Pb concentration (binomial generalized linear model, $p < 0.05$, Table S7).

It has to be underlined that soil Fe and pH (highly correlated: $r = -0.83$) did not significantly influence *N. caerulescens* growth in the models in the pH range of urban soils used in this study (6.2–8), which is narrower than previous studies (Maxted et al., 2007; Wang et al., 2006; Yanai et al., 2006) that highlighted a significant effect of pH on growth (from pH 4.4 to 7.8). Finally, none of the soil major cations (Ca, K, Mg and P) were selected to explain the variation in biomass production.

The two populations used in this study had similar total biomass production, but had a very different ratio of reproductive/vegetative biomass (R/V): GAN having a higher proportion of reproductive parts (R/V 0.54) than LUX (R/V 0.13). This is explained by the difference in dominant life cycles (annual for GAN, biennial for LUX), already shown in phytoextraction field trials (Jacobs et al., 2018b).

4.2. Soil influence on metal uptake

Accumulation levels were low compared to what is obtained in field trials on some of the same soils of origin (2 to 10 times less) (Jacobs et al., 2017, 2018a, b) but it can be explained by a shorter growth and a smaller soil volume in this pot experiment. The two populations showed the expected contrasting patterns of shoot Cd and Zn concentrations (Escarré et al., 2000; Jacobs et al., 2017; Meerts and van Isacker, 1997): higher shoot Zn in LUX plants and higher shoot Cd in GAN plants. The difference in Cd concentrations between populations was however not present on all soils and was less pronounced than expected considering the high potential for Cd accumulation of GAN (31 μg g^{-1} in GAN *vs* 22 μg g^{-1} in LUX). Soil factors limiting Cd bioavailability could be an explanation.

Exchangeable soil Cd and Zn were the main explanatory variables of Cd and Zn concentrations, respectively, in aboveground parts of *N. caerulescens*, as already highlighted in numerous studies (McGrath et al., 2006; Maxted et al., 2007; Yanai et al., 2006). This was expected considering the large variation of soil contamination in pots (0.2–14 mg Cd kg^{-1} and 30–3200 mg Zn kg^{-1} as exchangeable forms). However, other soil variables also influenced shoot metal concentrations. We confirm the negative effect of available NO_3^- (or NH_4^+ for Zn concentrations in GAN) on shoot concentrations of Cd and Zn in both populations shown by previous studies (Monsant et al., 2008; Xie et al., 2009; Jacobs et al., 2018a). This effect can be linked to a dilution of metals absorbed in larger plants (NO_3^- has a positive influence on shoot growth) but it could also be due to an increased shoot-root ratio (Roosens et al., 2003) in the presence of high N concentrations and hence to a reduced soil exploration by roots (Morris et al., 2017).

There was a negative effect of soil Zn and Pb on shoot Cd concentrations in LUX population but not in GAN. This is coherent with the difference in Cd accumulation mechanisms between ecotypes, GAN ecotype having higher gene copy number and level of expression of specific cadmium transporters (HMA4 and HMA3) than other MET or NMET populations (Craciun et al., 2012; Halimaa et al., 2014;

Lombi et al., 2001a; Roosens et al., 2003). Cd uptake by NMET is therefore more sensitive to competition with Zn.

Increasing gravel charge of soils had a negative impact on shoot Cd and Zn concentrations and uptake in LUX plants, which can easily be interpreted as a dilution of available metal mass in the pot when the gravel charge is high. The negative effect of the carbonate index (CARB, separating soils below and above pH 7) on Cd concentrations and uptake with GAN population can be linked to a lower Cd bioavailability with the higher carbonate content. This highlights that soil pH 7 is the threshold determining Cd bioavailability for *N. caerulescens* in these soils, as Maxted et al. (2007) also observed, and also explains why GAN did not accumulate more Cd than LUX on seven soils with pH > 7 (AN3, AN4, BEM, MAS, NAV, SOL6, WIE). Finally the slightly negative effect of Ca on Cd concentrations and uptake suggests a competition for uptake between Ca and Cd as already observed in the Prayon population, which has however a very different genetic background than GAN (Dechamps et al., 2005; Roosens et al., 2003; Zhao et al., 2002). Rees et al. (2015) suggested that the lower bioavailability of Ca induced by the addition of biochar could explain the simultaneous increase in Cd and Zn uptake by the population of Ganges.

4.3. Effect of nitrogen fertilization on biomass, concentrations and uptake

We tested the effect of adding environmentally sustainable inputs of two N fertilizers (40 mg N kg^{-1} as KNO_3 or NH_4NO_3) because it is among the easiest ways to improve soil chemical fertility and enhance shoot biomass. Previously, two tests of sustainable N fertilization with NH_4NO_3 (about 100 kg N ha^{-1} or 30 mg N kg^{-1}) on *N. caerulescens* yielded highly variable biomass gains (+15–80%) depending on the soil (Sirguey et al., 2006, Jacobs et al., 2018a). Other studies also suggest that there might be some preference of *N. caerulescens* for NO_3^- rather than NH_4^+ (Monsant et al., 2008; Schwartz et al., 2003; Xie et al., 2009). We therefore compared two sources of N.

Nitrogen fertilization had a positive impact on *N. caerulescens* biomass production only in soils with low initial N content (<25 mg N-NO_3 kg^{-1}) but the growth stimulation for these soils was rather limited (+15 and 23% on average for GAN and LUX populations, respectively). The two N fertilizers (KNO_3 or NH_4NO_3) gave the same biomass increase. On soils with high initial N content (>100 mg N kg^{-1}), N addition had a negative effect on biomass. We could speculate that N fertilization enhance fungal development as already observed in the field (Jacobs et al., 2018a), and supported by the slightly lower survival rate on the N treatments (82.5%) compared to the control (89%) (χ^2 test, $p < 0.05$). This would explain why beyond a certain threshold of initial N in the soil, the negative impact of adding synthetic fertilizers on plant disease is stronger than growth stimulation. Furthermore, the negative effect of NH_4NO_3 on Cd and Zn concentrations offset the small biomass gain obtained with fertilization, which is coherent with the results of Sirguey et al. (2006) with similar levels of fertilization. Overall, N fertilization had a neutral effect on the wide range of garden and wasteland soils used in this study. It can be concluded that N fertilization does not improve phytoextraction except on soils with very low N content.

5. Conclusion

This study shows that soil exchangeable Cd and Zn are the major soil drivers of Cd and Zn uptake by *N. caerulescens*. Beyond soil Cd and Zn concentrations, the secondary drivers of trace metal uptake are organic matter content and bulk density which stimulate plant growth (for a high OM content and low bulk density), and soil Cu exchangeable concentrations which influence biomass production negatively. This confirms the low tolerance of *N. caerulescens* to Cu at even lower Cu concentrations (100–240 mg kg^{-1}) than previously demonstrated. The negative effect of high soil Zn content on Cd uptake in NMET population (LUX), but not in GAN, is also highlighted on field contaminated soils, while Cd uptake by GAN is negatively impacted by the carbonate content of the soil. Finally, we show that N fertilization at environmentally acceptable doses is not an efficient way of improving phytoextraction efficiency. This stresses the need to characterize extensively soil determinants for a better predictability of phytoextraction, as excessive Cu exchangeable concentrations, low OM content or high carbonate content can clearly impact negatively the extraction efficiency. Our study shows that extraction efficiency is best for moderate soil contamination in Cd (2–10 mg kg^{-1}) and Zn (300–1000 mg kg^{-1}).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.02.073.

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