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Differential elemental uptake in three pseudo-metallophyte C_4 grasses in situ in the eastern USA

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Abstract

Background and aims—Elemental uptake in serpentine floras in eastern North America is largely unknown. The objective of this study was to determine major and trace element concentrations in soil and leaves of three native pseudo-metallophyte C_4 grasses in situ at five sites with three very different soil types, including three serpentine sites, in eastern USA.

Methods—Pseudo-total and extractible concentrations of 15 elements were measured and correlated from the soils and leaves of three species at the five sites.

Results—Element concentrations in soils of pseudo-metallophytes varied up to five orders of magnitude. Soils from metalliferous sites exhibited higher concentrations of their characteristic elements than non-metalliferous. In metallicolous populations, elemental concentrations depended on the element. Concentrations of major elements (Ca, Mg, K) in leaves were lower than typical toxicity thresholds, whereas concentrations of Zn were higher.

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Conclusions—In grasses, species can maintain relatively low metal concentrations in their leaves even when soil concentrations are richer. However, in highly Zn-contaminated soil, we found evidence of a threshold concentration above which Zn uptake increases drastically. Finally, absence of main characteristics of serpentine soil at one site indicated the importance of soil survey and restoration to maintain serpentinophytes communities and avoid soil encroachment.

Keywords

C_4 grass; accumulation; excluder; Serpentine; Calamine; Pseudo-metallophytes

Introduction

Metal and metalloid trace elements have variable concentrations in parental bedrock that define natural background concentrations in soils (Gao et al. 1998; Alloway 2013). Based on variation in parent rock composition and alteration processes, some soils contain elements at concentrations well above global median values (Kabata-Pendias 2000). For example, serpentine soils derived from ultramafic bedrock is richer in Ni and Cr than other soils (Massoura et al. 2006; Alloway 2013); likewise, concentrations of Cd and Zn also vary with underlying bedrock type (Jyoti et al. 2015). Trace metals may also be elevated from anthropogenic sources such as smelting, military or agricultural activities (Alloway 2013). Indeed, metalliferous sites can be characterized as primary, secondary, or tertiary, based on whether soil metals occur naturally, are resulted from mining activities, or were deposited as pollution from smelters or other sources, respectively. Sites can also be classified by the particular elevated elements, which are mainly calamine (Cd, Pb and Zn), copper-cobalt outcrops (Faucon et al. 2016) and serpentine (Cr, Co, Ni) in temperate climate (Baker and Brooks 1989).

Metals in soils can have consequences for vegetation. At high concentrations, as in serpentine soils, trace elements can induce phytotoxicity and reduce plant fitness (Clemens 2001; Lin and Aarts 2012). Many species found in metalliferous sites, called metallophytes, are able to cope with elevated metal concentrations and form unique, endemic plant communities (Baker et al. 2010; Faucon et al. 2016; Isnard et al. 2016). Some plants called pseudo-metallophytes have the ability to grow on both metalliferous and non-metalliferous soils (Pollard et al. 2002; Pollard et al. 2014).

In the eastern US, many ultramafic outcrops are found along the Appalachian orogenic belt from Québec and Newfoundland south to Georgia and Alabama (Rajakaruna et al. 2009; Alexander 2009). These areas exhibit common soil properties of the “serpentine syndrome”: high Cr, Co and Ni concentrations in soil, Ca:Mg ratio below a value of 1.0, and deficiency of essential macronutrients (Brady et al. 2005; Kazakou et al. 2008). Serpentine sites are also characterized by poor plant productivity, high rates of endemism and distinct vegetation from those of neighboring areas (Whittaker 1954 in Brady et al. 2005). Most serpentine sites, termed serpentine barrens, in eastern North America were under ice during the last glaciation (Last Glacial Maximum, 23,000–15,000 years ago, Prentice et al. 1991; Williams 2003), which means they represent a relatively new ecosystem without much time for plant species divergence, but possibly for ecotypic variation (Burgess et al. 2015b). Compared to similar soils in unglaciated sites across the world, Eastern serpentine sites present only two endemic species (Harris and Rajakaruna 2009; Boufford et al. 2014), namely *Adiantum viridimontanum* (Pteridaceae) and *Minuartia marcescens* (Caryophyllaceae), whereas the Western serpentine sites contain more than 246 endemic species among 670 species (Anacker et al. 2011).

The serpentine plant community is mainly characterized by patches of typical grasslands or savannas, as islands embedded within Eastern deciduous forest. Disturbances such as mowing, mining, wildfires, and grazing help maintain grasslands or savannas on Eastern serpentine by prohibiting plant succession to forest (Latham 1993; Arabas 2000; Burgess et al. 2015a). Although Eastern serpentine may contain patches of unusual woodland and wetland communities, these sites are dominated by grasses, particularly several C_4 grasses that are also major components of tallgrass prairie of the Great Plains in Midwest Region (Bever et al. 1996; Ji et al. 2013). Despite the importance of this grass species on serpentine outcrops, little has been investigated about their metal uptake properties in part, because no known hyperaccumulator species are found there (Rajakaruna et al. 2009).

Tolerant metallophytes exhibit alternate adaptive traits enabling them to either resist uptake or detoxify trace elements within their tissues (Baker 1981; Baker 1987). They present three different patterns of metal homeostasis: excluders maintain low concentrations in above-ground tissue over a large range of soil concentrations, indicators show similar concentrations as soils, and hyperaccumulators concentrate trace elements in above-ground tissue parts independent of soil levels (Baker 1981; Van der Ent et al. 2013; Pollard et al. 2014). To date, research has focused on hyperaccumulators because they hold promise for detoxifying metal-polluted soils through phytoremediation–mainly phytoextraction and phytomining (Cunningham et al. 1995; Ali et al. 2013; Van Der Ent et al. 2015).

Learning more about the metal uptake properties of other tolerant species, especially grasses, is important because of their utility in stabilizing and revegetating metal contaminated areas (Chaney et al. 1997; Li et al. 2000), which can be new environments for these plants. A good example is the revegetation of the superfund site called Palmerton, a calamine site managed by the Le-high Gap Nature Center. The north-facing slope of the mountain was contaminated with chalcophile elements (cadmium, lead, and zinc) due to emission from two zinc smelters operated from roughly 1898 until 1980. Revegetation of the site began in 2002. Our samples for this study were collected in an area that received aerial application of fertilizer, lime, and a mixture of different cultivars of grass seeds (Latham et al. 2007a), including several C_4 grasses. Currently, information about elemental concentrations are scare and come from greenhouse experiments (Glassman and Casper 2012). Not all elements and their interaction were determined.

To date, we know of no comprehensive study that examines uptake for a suite of metals in a native grasses species in metalliferous (serpentine and calamine) and in non-metalliferous sites. Since pseudo-metallophytes can still show higher metal concentrations on metalliferous soils, they can cause metallicolous and non-metallicolous congeneric populations to be considered sub-species (Boyd and Martens 1998) or simply different ecotypes of the species or edaphic type (Pauwels et al. 2005; Gonneau et al. 2014; Burgess et al. 2015b).

We aim to examine variation and correlation of major and trace element concentrations in soils from different sites that include three soil types: serpentine (primary and secondary metalliferous), calamine (tertiary metalliferous), and non-metalliferous site. We expected that concentrations of characteristic elements for two types of metalliferous sites were higher than in the non-metalliferous site and with higher variation of the other elements. We also examine the variation associated with individuals (local variation) and species within site for all elements. Furthermore, we compare metal uptake in the three native grasses, which are considered to be metal excluding plants, across the different soils. To achieve these objectives, we measured trace element concentrations in soils and in aboveground plant tissues in sites. The three species we target, *Andropogon gerardii*, *Schizachyrium scoparium*, and *Sorghastrum nutans*, are dominant C_4 grasses in Eastern North America. We sampled these grasses from three serpentine grasslands, one calamine site polluted by nearly 100 years of zinc smelting, and one non-metalliferous site where land management practices maintain a grassland.

Materials and methods

Site studies and sampling

In fall 2014, leaves (less than two grams) and rooting soil (approximatively 1.0 l using a stainless steel shovel) from the same plants were collected from five sites corresponding to three edaphic type: serpentine group (SERP) with three sites, calamine (CAL, one site), and nonmetalliferous (NMET, one site). The serpentine sites are located in Chester County, PA (Rajakaruna et al. 2009): Nottingham (NOT, 39°44″8.53″N 76° 2″9.94″ W) in Nottingham County Park; Sugartown (SGW, 40°00″22.7″N 75°31″56.7″W) within the boundaries of Willisbrook Preserve; and Unionville (UNI, 39°55″ 15.2″N 75°43″30.9″W), mostly within the boundaries of the ChesLen Preserve. The calamine site is located on Blue Mountain, near the town of Palmerton in Carbon County, PA (PAL, 40°47′9.60″N 75°37′8.40″W) The non-metalliferous site is located at Fort Indiantown Gap in Lebanon County, PA (FIG, 40°26′24.2″N 76°34′53.15″W). It is a military training area managed by the Pennsylvania National Guard (Latham et al. 2007b; Ferster et al. 2008). Grassland vegetation is maintained by removing woody vegetation mechanically or by fire.

At each site, we collected eight individuals with at least 30 m fair away for each other of each of three C_4 grass species: *Andropogon gerardii* (*A. ger*), *Sorghastrum nutans* (*S. nut*), and *Schizachyrium scoparium* (*S. sco*). Due to loss during the drying process, the soils from Palmerton (PAL) were analyzed for five individuals of each species.

Plant and soil analysis

Leaf samples were cleaned three times tap water and one time with ultrapure water (18 mΩ.cm), dried for 42 h at 50 °C, and crushed into small pieces according to Rees et al. (2015). Crushed leaves (0.5 g) were digested with a mixture of HNO_3/H_2O_2 using a DigiPrep system (SCP science, USA) following a method outlined in Gonneau et al. (2014).

For N measurement, approximately 0.25 g of fine ground material (< 200 μm) was weighed into a tin capsule (Elemental Microanalysis, Okehampton, Devon, UK) on a microbalance. Analysis of N concentrations was conducted by combustion using Vario Max C/N analyser (Elementar, Germany).

All of the soils were dried at 65 °C for 48 h and sieved to remove grains with size above 2.0 mm. Soil pH was measured from a solution consisting of 1:5 ratio of soil to ultrapure water. Pseudo-total concentrations (an assessment of the maximum potentially soluble element and not bound in silicates) of major elements (Al, Ca, Fe, K and Mg) and trace elements (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) were determined by EPA method 3050, which involves digestion of 1.0 g ground soil (< 200 μm) in aqua regia (mixture of $HNO_3/H_2O_2/HCl$) in a DigiPrep system.

The soil extractible quantity of each element, the fraction considered available to plants, was measured as follows. Phosphorus was extracted by mixing 1.0 g of soil with a solution of 0.025 N HCl and 0.03 N NH_4F (Bray-1) for 5 min. The amount of phosphorus was determined by colorimetric analysis at 880 nm (Hach, DR 6000, USA). To extract other elements, we used two solutions: DTPA (Al, Cd, Co, Cr, Cu, Mn, Ni and Pb), and ammonium acetate (Ca, K, Mg, Na). For the DTPA extraction, 10 g of soil was mixed in 20 mL of 0.005 M DTPA +0.01 M $CaCl_2$ + 0.01 M TEA at pH 7.3 for 2 h (Lindsay and Norvell 1978). For the ammonium acetate extraction, 1 g of soil was mixed in 10 mL of 1 M ammonium acetate (NH_4OAc) solution at pH 7.0 for 2 h (Thomas 1982) to determine cation-exchange capacity (CEC) and Ca, K, Mg, and Na. All solutions were filtered through a 0.45 μm membrane (nylon) before measuring the elemental concentration using inductive coupled plasma optical emission spectroscopy (ICP-OES, Spectro Genesis, Spectro Analytical Instruments, Germany). For each analysis, the instrument was calibrated using matrix-matched element standards, and ten blanks were used to determine quantification limits. A control solution (EuH_4, SCP science, USA) was also analyzed to verify accurate values.

Data processing

All statistical analyses were performed using R v2.10.14. Before analysis, all data were log-transformed in order to improve normality and variance homogeneity of residuals. All parameters were analyzed by a nested two-ways analysis of variance with site and species nested within site followed by post-hoc analysis. Variance components were estimated for site, species within site, and samples within species within site. Variance components were estimated for each factor using the restricted maximum likelihood (REML) method with the lmer function inside the lme4 package. Constrained Analysis of Proximities (CAP), a constrained ordination technique) was used to examine the relationships between plant species identity, site and soil chemical characteristics using “capscale” command in the “vegan” package in R. Firstly, CAP analysis was performed on the pseudo-total concentrations in rooting soil samples and secondly for extractible concentration of elements and pH. A third CAP was used to examine elemental concentrations in leaves. For each CAP analysis, the permutation-based “anova.cca” method was performed to test the significance of site and species within site as a predictor of plant and soil chemistry, and the “plot.cca” method to visualize the results. Finally, for each edaphic group (SERP, CAL and NMET), multiple correlation tests between all soil and leaf parameters were performed using the Pearson method and FDR control for type I errors. Based on our analyses for extractible quantities of elements, we separated collections at the SGW serpentine site into different edaphic groups. We grouped collections under *S. scoparium* with those from NOT and UNI but made *A. gerardii* and *S. nutans* a group of their own.

Results

Variation of soils properties

All pseudo-total concentrations of major and trace elements showed significant differences between sites and, for some elements, differences between species (Table 1). Pseudo-total and extractible concentrations of elements in rooting soil varied from one to five orders of magnitude (Tables S2 and S3). Percentage of variance associate from sample to site factor varied from 66.7% for Fe to 100% for Zn (Table S1). Sites more often explained a greater percentage of variance than species within site (Table S1).

CAP analysis with all pseudo-total concentrations showed clear distinction of three types of soil based on trace elements concentrations but not a clear separation by species under which the soil was collected (Fig. 1a). Both site and species within site effect were highly significant ($p < 0.001$). CAP1 and CAP 2 explained 71.7% and 24.5% of variance respectively. Three main part were observed with (i) PAL, the calamine site, showed higher pseudo-total concentrations of Cd, Pb, and Zn than other sites (Table S2 and S3), (ii) FIG, and (iii) three serpentine sites, displaying the highest concentrations in Co, Cr and Ni. Compared to the two other serpentine sites, SGW had lower concentrations of Co and Ni (but not Cr).

Pseudo-total concentrations of other elements (Al, Ca,Cu, Fe, K, Mg and Mn) much variation in the CAP analysis or contribute to the separation of sites by edaphic group (Table S2 and S3)., Finally the Ca:Mg ratio in rooting soil in serpentine was characteristically lower than 1.0 and much lower than in FIG and PAL (Table S2). Extractible soil concentrations of all elements except K differed between sites (Table 1). Soil pH was similar between sites (Table S3). The total percentage of variance explained by site, species within site, and samples within species within site ranged from <10% for Cu and K to >90% for Al, Fe, P, Mn, and Zn (Table S1). The percentage of variance explained by site varied more among elements than the percentage of variance explained by species within site (Table S1). Concentrations of Cd, Cr and Pb were below detection limit for most individuals except in PAL (Table S2 and S3).

For extractible quantities of elements, CAP analysis likewise, showed significant differences according to site and species within site (Fig. 1b). CAP1 which explained 52.4% of the variance was negatively correlated with Ca:Mg ratio and positively correlated with Ni and Mg, thereby separating serpentine sites from FIG and PAL (Fig. 1b). Extractible concentrations of Cd, Pb and Zn were negatively correlated with the CAP2 axis, which explained 36.4% of the variance. These elements are highest in soils from PAL. Finally, soils from FIG and SGW were separated from NOT and UNI on this axis. Among species, *A.*

gerardii and *S. scoparium* showed the greatest separation while *S. nutans* overlapped with both (Fig. 1b).

Examining extractible quantities of elements individually, Al, Ca, Cd, Cu, P, Pb and Zn were higher in PAL (Figs.2 and 3, Table S3). Mg concentrations were higher in NOT and UNI than in SGW and FIG (Fig.2). Ni concentrations were higher in NOT and UNI than in SGW and then in FIG and PAL (Fig. 2b). Al, Ca, Cu, and Mn differed also among species within sites (Table S2). At SGW *A. gerardii* and *S. nutans* showed higher Ca:Mg ratio (2.40 and 1.76, respectively) than *S. scoparium* (0.368) (Table S3); Ca:Mg ratio below 1.0 are typical on serpentine soil and are thought to range from 0.238 to 0.596. At FIG, soils under *S. nutans* and *S. scoparium* were more acidic (average pH 5.82 and 5.73 respectively) than soils under *A. gerardii* (average pH 6.86, $p < 0.001$).

Element concentrations in leaves

Concentrations in leaves differed among sites and species for many elements (Table 1). The total percentage of variance explained by site, species within site, and samples within species within site was less than 25% for K, Co, Cr, and Ni and greater than 90% for Al, Cu, Mn, N, N:P ratio and Pb (Table S1). For Al, Cu, Mn, N, N:P ratio and Pb, most of variance was explained by samples. Site explained a higher percentage of variance for Ca, Fe, Mg, Ca:Mg ratio, and Cd. Species within site explained a higher percentage of variance only for P.

Site and species within site were also significant by CAP analysis ($p < 0.001$; Fig. 1c). CAP1, which explained 82.7% of the variance, was negatively correlated with Ca and positively correlated with Mg (Fig. 1c). This axis separated the FIG site in the Ca direction and UNI/NOT in the Mg direction. CAP2, which explained 10.1% of the variance, was negatively correlated with Zn, separating PAL from the other sites. Species showed clear separation without an overlap, with a greater difference between *A. gerardii* and *S. scoparium* (Fig. 1c).

Consistent with serpentine syndrome, plants from the serpentine sites had higher Mg concentrations and lower Ca concentrations and Ca:Mg ratios (Table S4 and Fig. 2c). Only N, Co and Ni in leaf tissue did not vary between sites (Tables 1 and S4, Fig. 3). Cd, Pb, and Zn were higher in the calamine site PAL (Table S4 and Fig. 3). Concentrations of Cd were below 10 mg kg-1 and concentrations of Zn reached 1000 mg kg^{-1} at PAL (Fig. 3). Concentrations of Cd and Zn were below 5 mg kg^{-1} and 50 mg kg^{-1} in the other sites respectively (Fig. 3). Other elements did not present particular patterns by edaphic type (Table S4). N:P ratio were higher in NOT and UNI ($N{:}P = 12$) than three others sites with $N{:}P < 9$ (Table S4). Major elements showed more differences between species than did trace elements. Within sites, the greatest differences between three species were found at SGW with lower concentrations of Ca and Ca:Mg ratio for *S. scoparium*, lower concentrations of Mg and Na for *S. nutans*, and higher concentrations of Pb for *A. gerardii*. At UNI, *S. scoparium* showed higher concentrations of Cu than *S. nutans*, and at PAL *A. gerardii* showed the lowest concentrations of Mn (Table S4).

Correlation within soil and leaf parameters

Many of the concentrations for different elements were significantly correlated ($p < 0.05$), both in leaves and in rooting soils for four group from CAP analysis based on pseudo-total and extractible concentrations (Fig. 4 and S1). In order to describe the strongest correlations, only |r| values above 0.5 were taken into account.

On this basis, some positive correlations among elements measured in leaves, such as Co, Cr and Pb, were very similar among the four groups (Fig. 4). K and P were positively correlated except in serpentine group. Al and Fe were positively correlated at PAL and FIG. In the serpentine group and FIG, there were positive correlations between Al and Co, Cr and Pb and Cu and Pb. There were positive correlations between K and Mn and between Mg and P for *S. nutans* and *S. scoparium* at SGW and for PAL. A positive correlation between Co and Zn, Cr and Zn, Mn and Zn was found only for FIG and for *S. nutans* and *S. scoparium* at SGW. Some of the strongest correlations between elements were found only in one group namely between Cu, Pb and Zn at FIG or between major elements such as Ca, K, N and P for individuals of *S. nutans* and *S. scoparium* at SGW. At PAL, Cd was postively correlated with Pb and Zn. A negative correlation between elements was less common. For *S. nutans* and *S. scoparium* at SGW Cd was negatively correlated with Co, Cr, Pb and Zn (Fig. 4b). At PAL, Ca and Mn were negatively correlated, and Mg and P were both negatively correlated with Cu and Zn (Fig. 4c).

Correlations between elemental concentrations in leaves with either pseudo-total or extractible soil concentrations were mostly weak or non-significant. Among the strong relationship between levels in soil and plant, were positive correlations in the serpentine group for Ni and Zn, a positive correlation for Mg and negative correlations for Cu and Pb at PAL, and a positive correlation for Ca at FIG (Table S5).

Pseudo-total concentrations of elements in soils were mainly positively correlated between them (Fig. S1). Pseudo-total concentrations of siderophile elements (Co, Cr, Ni and Mn) were positively correlated in soils. The exception was for Mn at PAL (Fig. S1). Major elements showed mostly positive correlations between pseudo-total and extractible concentrations. Except for a few cases, extractible elements were less correlated than pseudo-total concentrations. Trace elements, for example, showed lower correlations for FIG and PAL compared to serpentine sites. Cd and Zn were positively correlated for Palmerton for pseudo-total and extractible concentrations (Fig. S1).

Discussion

In this study, we evaluated characteristic element concentrations and their variability in rooting soil and in leaves of the three dominant C_4 grasses (*Andropogon gerardii*, *Schizachyrium scoparium*, and *Sorghastrum nutans*). Soils properties showed important variability and not only for elements expected to be high in two metalliferous edaphic group. Results showed the importance of the measurement of many elementals in rooting soil since soil at SGW showed non-serpentine properties even if parental bedrock seems to correspond to this edaphic type. Three C4 species are tolerant to metalliferous soils properties and generally showed low trace element concentrations in leaves despite a wide range of concentrations in non-metalliferous to metalliferous soils. Only Zn, at a contaminated site, was considerably elevated in leaf tissue.

Soil composition under pseudo-metallophyte species

The metal composition of soils under the three C_4 grasses were clustered more by the edaphic group and by site than by species identity, suggesting no spatial sorting of species by soil characteristics. The metalliferous sites studied here showed higher concentrations of trace elements than did the non-metalliferous site by one to five orders of magnitude. Pseudo-total concentrations of most trace elements in rooting soils were within the range of values found in 4857 soil sampling sites distributed in the contiguous United States (Smith et al. 2013; Woodruff et al. 2015). Concentrations in two metalliferous types of soils were mostly higher than the third quartiles of those samples, indicating metalliferous soils characterized by high concentrations.

We found three main distinguishing characteristics of ultramafic soils at two of three serpentine sites, NOTand UNI: elevated concentrations of some trace elements (especially nickel, cobalt, chromium), a Ca:Mg ratio < 1, and deficiency in essential plant nutrients like phosphorus, but not K (Brady et al. 2005; Massoura et al. 2006; Kazakou et al. 2010). While P concentrations in three serpentine sites did not differ from the non-metalliferous native grassland FIG (Table S3), P, measured by the Bray-1 method, was at least four to ten times lower than reported values for two non-metalliferous native grasslands in the Great Plains, Firmi and Cedar Creek where the focal grasses occur (Johnson et al. 2010). Similar differences were observed for P, measured using the Melich-3 method, among soils collected under the same species in East Coast serpentine sites and two other grasslands in Iowa (Ji et al. 2012); such differences were not found for NH_4-N and NO_3-N.

Due to their similar origin and affinity to iron (Goldschmidt 1937), relatively high pseudo-total concentrations of Ni, Co, and Cr, which were higher in serpentine, are found together in soils. Extractible concentrations of Ni in serpentine soils were one order of magnitude higher than in others sites, but there was less of difference between serpentine and others sites for Cr and Co. Cr is not detected by DTPA extraction, as it is mainly included in resistant mineral phases, which do not dissolve significantly during this extraction. Indeed, both extractible quantities and the mobility of these three elements differ. Analyzing the mobility of Ni, Cr, and Co in six serpentine sites in Poland, Kierczak et al. (2016) showed that the highest mobile element was Ni whereas Cr was the least mobile.

The Ca to Mg ratio was low (<0.3) in most serpentine soils except under *S. nutans* and *A. gerardii* at SGWand typically lower than 1 (Brady et al. 2005; Rajakaruna et al. 2009). Still the values were higher than reported for other serpentine sites in the eastern US such as in Maryland (0.03–0.15; Alexander 2009) and more similar to some serpentine sites in France or in the eastern Europe (Massoura et al. 2006). Compared to most serpentine outcrops elsewhere, serpentine sites in northeastern regions of North America were previously glaciated, and deposits by made by moving glaciers may have ameliorated some aspects of serpentine soils detrimental to plant growth (Rajakaruna et al. 2009). Alternatively, these sites present inherently different mineralogy and serpentinization process.

At Palmerton, the calamine site, Cd and Zn concentrations in some soil samples were close to high values found just after smelting ceased and before revegetation (Brown et al. 1994; Li et al. 2000) but lower than some concentrations in some localities on this mountain with 40,000 mg kg^{-1} (Palazzo et al. 2003). In all case, observed values here exceeded the maximum values recorded in another survey of contaminated areas in the US (76.8 and 1700 mg kg^{-1} respectively, Woodruff et al. 2015). Smelting activities, like those at Palmerton, represent the main source of pollution among industrial sources, with a large increase and enrichment of trace elements in the environment compared to geogenic concentrations (Nagajyoti et al. 2010). Cd and Zn extractible concentrations were closed to concentrations observed in another superfund site, Callahan Mine in Brooksville, ME, USA (Mansfield et al. 2014). However, our measured Cd and Zn pseudo-total concentrations were 10 and 15 times lower, respectively, than concentrations observed in other calamine sites occurs in the south of France (mining waste pile) and in Belgium (smelter activities) (Bert et al. 2002; Escarré et al. 2011).

Trace element concentrations at FIG were below detection limits or within the range of values found for non-metalliferous soils in the US (Smith et al. 2013). Nevertheless, a few samples under *S. nutans* at FIG showed high Pb pseudo-total concentrations (50–300 mg kg^{-1}). These soils presented a different relationship with Fe compared to the other soils at the same site indicating a local increase of Pb, likely from anthropogenic sources (Fig. 1). This specific area corresponds to a major military shooting range (Ferster et al. 2008). Concentrations is higher than concentrations observed in mining site in Callahan Mine in Brooksville, ME, USA (Mansfield et al. 2014) but lower than some mining site in Southern of France where Pb hyperaccumulator occurs (Escarré et al. 2011). Lead is a major component of bullets, and the abrasion of Pb bullets passing through soil could result in contamination with smaller metallic Pb particles and may have contributed to the total content of soil Pb content (Astrup et al. 1999; Bennett et al. 2007). Due to its relatively low solubility, Pb has a long residence time in soils. Robinson et al. (2008) reported variable Pb concentrations in a Swiss military shooting range with higher maximum concentrations (> 1000 mg kg^{-1}) compared to FIG.

Evaluating elemental uptake in excluder strategies

The elemental profiles in leaves of the three C_4 grasses studied here qualitatively mirror variation in the soil. Moreover, differences in leaf concentrations of Ca and Mg concentrations between serpentine and non-serpentine sites structured CAP analysis. The mean Ca concentration was below 2500 mg kg^{-1} for serpentine populations of *S. scoparium* and *Sorghastrum nutans* at NOT and UNI, but the mean concentration of Mg exceeded the deficiency threshold (2000 mg kg^{-1}). Both Ca and Mg concentrations presented here were much lower than concentrations found by Kazakou et al. (2010) in 21 plant species, including dicots, collected in situ at four serpentine sites in Lesbos Island but within the range of concentrations for 14 different vascular plants at a serpentine site in Maine, USA (Pope et al. 2010). Different reports demonstrated that grasses have a lower Ca requirement than dicots because their Type II cell walls are composed of cellulose fibers encased in glucuronoarabinoxylans and contain high levels of hydroxycinnamates with very low levels of pectin and structural proteins (Vogel 2008; O'Dell and Rajakaruna 2011; Marschner

2012). The Ca:Mg ratio < 1.0 found in leaves for our study populations resulted from lower concentrations of Ca. These results are consistent with the tolerance of Ca deficiency hypothesis and/or Mg toxicity proposed by Kruckeberg (1954 in Kazakou et al. 2008). For other major, essential elements, the C_4 grasses presented concentrations near those considered deficient for many grasses or other crops (Marschner 2012). Indeed, most plants presented Fe, P and K concentrations below 100, 2000 and 15,000 mg kg^{-1} respectively. These findings are similar to those of Pope et al. (2010) who sampled 14 species at a serpentine site in Maine and showed that some Poaceae presented lower than 1000 mg kg^{-1}. The N:P ratio, another important stress indicator, is within the normal range [10, 20] (Güsewell 2004) for all plant samples from the serpentine group except for *A. gerardii* and *S. nutans* at SGW. However, lowest N:P ratio in three species at PAL and FIG reflect N limitation for last sites.

For trace elements, our results indicate that the C_4 grasses are excluder species and can maintain consistently low trace element concentrations in aboveground tissues over a wider range of soil concentrations. Reduced elemental concentrations in leaves, result from different mechanisms, including the sequestration of most trace elements in the roots, restricting uptake and transport to the shoots (Baker 1981) and influences of soil microbe (Doubková et al. 2011). Any such mechanism failed only for Zn at Palmerton because concentrations in leaves are at least ten times higher than in others sites, suggesting a potential break point or critical level of Zn in soil where homeostasis breaks down. Due to a large difference in Zn concentrations, for both pseudo-total and extractible concentrations, between Palmerton and the four other sites, we did not identify the breaking point in our study. Indeed, Zn, and to a lesser extent Cd, concentrations in leaves at Palmerton were higher than the toxicity threshold for most plants (Krämer 2010). Grasses cultivated on soil from PAL in the greenhouse show high concentrations of Zn in their aboveground tissues (Li et al. 2000; Palazzo et al. 2003), and there is evidence that soil microbes influence levels of Zn in C_3 grasses from the site (Glassman and Casper 2012) and *S. nutans* (Casper, unpublished data). Mans-field et al. (2014) found that two species in the Salicaceae showed concentrations higher than 1000 mg kg^{-1} at the Callahan Mine in Brooksville, ME, USA. Indeed, it is usual to observed high concentrations of Zn in aboveground tissues in the plant families Brassicaceae, Poaceae or Salicaceae (Broadley et al. 2007). Broadley et al. (2001) found significant variation in shoot metal content occurred at the level of order and above, suggesting an ancient evolution of this trait.

In contrast, Ni concentrations were similar for plants of the same species at different sites despite important variation between sites in soil Ni levels. The availability of Ni in soils and its subsequent transfer to plants depends on its mineralogical origin (Kabata-Pendias 2000; Massoura et al. 2006) as well as on soil characteristics such as pH, conductivity, organic matter and clay content (Kabata-Pendias 2004), and even plant species identity. Using Ni spiked soil, Doherty et al. (2008) found that Ni concentrations in leaves of *S. nutans* were similar across seed and microbial communities from serpentine and non-serpentine origin, and was marginally affected by Ni dose. Other studies have shown differences between plant species in Ni content (Baker 1981; Kazakou et al. 2010; DeHart et al. 2014).

Finally, species accounted for more variation in leaf element concentrations than they did for soil element concentrations, suggesting species-specific differences in homeostasis in response to soil properties. Thus, foliar elemental profiles of *Schizachyrium scoparium* were characterized by relatively high concentrations of Co, Cr, Cu and Ni whereas *Andropogon gerardii* and *Sorghastrum nutans* showed higher concentrations of Ca at PAL and K at FIG. Leaf concentrations represent the product of many factors including soil properties as described above and several potential filtering processes in roots, including metal efflux from the plasma membrane (Curie et al. 2009), metal chelation by phytochelatins and metallothioneins (Schat et al. 2002; Devez et al. 2009), and compartmentalization within the vacuole (Shaw et al. 2005). Even in species known to exclude trace elements, Kazakou et al. (2010) found higher Mg and Cr concentrations and lower Ca to Mg ratios in plants from serpentine soils compared to non-serpentine soils.

Phytostabilization and site management strategies

Much research focuses on using hyperaccumulators to rehabilitate metal-contaminated land through phytoextraction (Hammer and Keller 2003; Broadhurst et al. 2015) and phytomining (Bani et al. 2007; Van Der Ent et al. 2015) and metallophytes are the optimal choice for vegetation in restoration ecology and remediation in mining sites. Phytostabilization represents another way to remediate, especially in soils with high trace element concentrations like those at industrially contaminated sites The three C_4 grasses studied here are present across a range of soil types including those with high concentrations of Pb and Cd, which are considered hazardous in soil (Järup 2003). With low metal concentrations in leaves and the absence of any correlations between metal levels in soils and plants, members of the Poaceae are excellent candidates for phytoremediation of the many Pb contaminated areas found across the northern United States (Burt et al. 2003; Woodruff et al. 2015). The could also be used for phytostabilization of serpentine sites, areas contaminated with Ni by industrial activities, or asbestos quarries (Rodríguez-Seijo and Andrade 2015).

Elemental concentrations in soils under *S. nutans* and *A. gerardii* at Sugartown (SERP) were closer to non-metalliferous soils than soils under *S. scoparium* in same site and were spatially separated from *S. scoparium* (C. Gonneau, *personal observation*). These soils properties indicate loss of the serpentine syndrome (Kazakou et al. 2008) and a shift to non-serpentines conditions approximatively since 100 years ago (Burgess et al. 2015a). When bordering woodlands encroach on grasslands they create a richer soil layer over the serpentine soils as their leaves drop and decompose (Haegele 2011), alter the mycorrhizal community (Cumming and Kelly 2007), and lead to the disappearance of a particular and unique flora and fauna (Rajakaruna et al. 2009; Latham and McGeehin 2012). The tallgrass prairie is one of the most critically endangered ecosystems in North America mainly due to the invasion of woody plants encroachment (Hoekstra et al. 2005). Mesophication is also a main problem for grassland, with growing dominance by Acer, Nyssa and more mesic Quercus and Fagus species (Burgess et al. 2015a). To sustain this species on many serpentines areas, a Restoration and Management Plan is implemented, which includes prescribed burning and trees and alien species removal as well the soil organic matter (Latham et al. 2007a; Gustafson et al. 2008; Latham 2008). The establishment of state law to protect this extreme environment is also important (O’Dell 2014) since serpentine site represent a globally rare ecosystem and a cluster of rare species give the site national or even global significance (Baker et al. 2010; Mengoni et al. 2010). Understanding plant metal uptake in grasses and their distribution in serpentines sites can inform better management strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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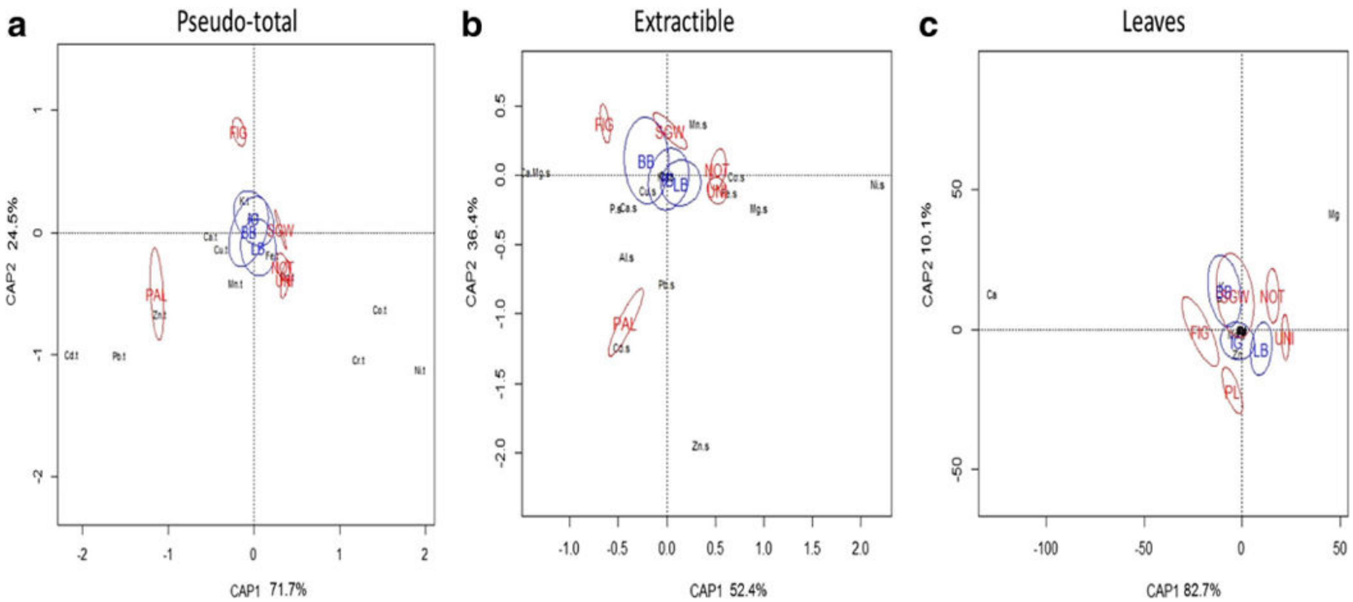

Fig. 1.

CAP ordination space of elemental concentrations in **a** pseudo-total, **b** extractible and **c** in leaves with position of species in blue and site in red. NOT: Nottingham, SGW: Sugartown, UNI: Unionville, PAL: Palmerton and FIG: Fort Indiantown Gap

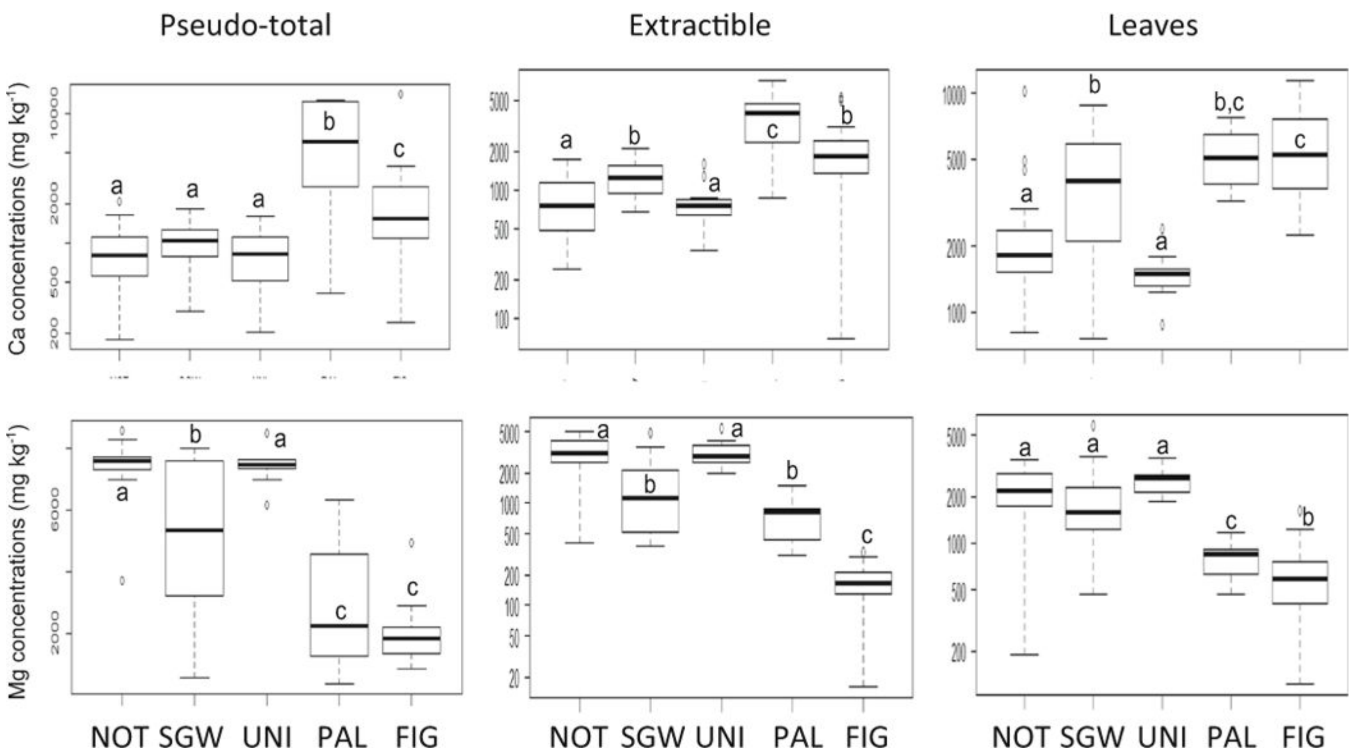

Fig. 2.

Concentrations of Ca and Mg (mg kg^{-1}) in five sites in ENA: pseudo-total, extractible and in leaves. NOT: Nottingham, SGW: Sugartown, UNI: Unionville, PAL: Palmerton and FIG: Fort Indiantown Gap

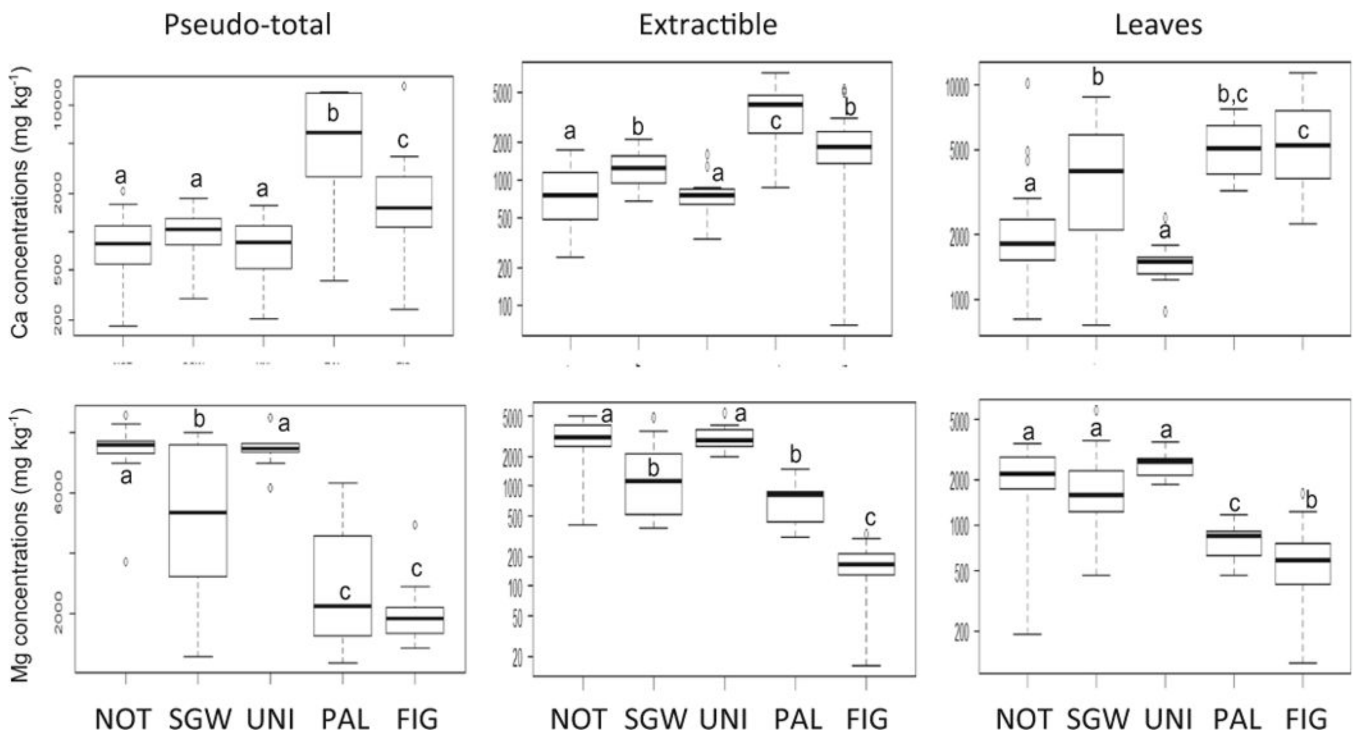

Fig. 3.

Concentrations of Ni and Zn (mg kg-1) in five sites in ENA: pseudo-total, extractible and in leaves. NOT: Nottingham, SGW: Sugartown, UNI: Unionville, PAL: Palmerton and FIG: Fort Indiantown Gap

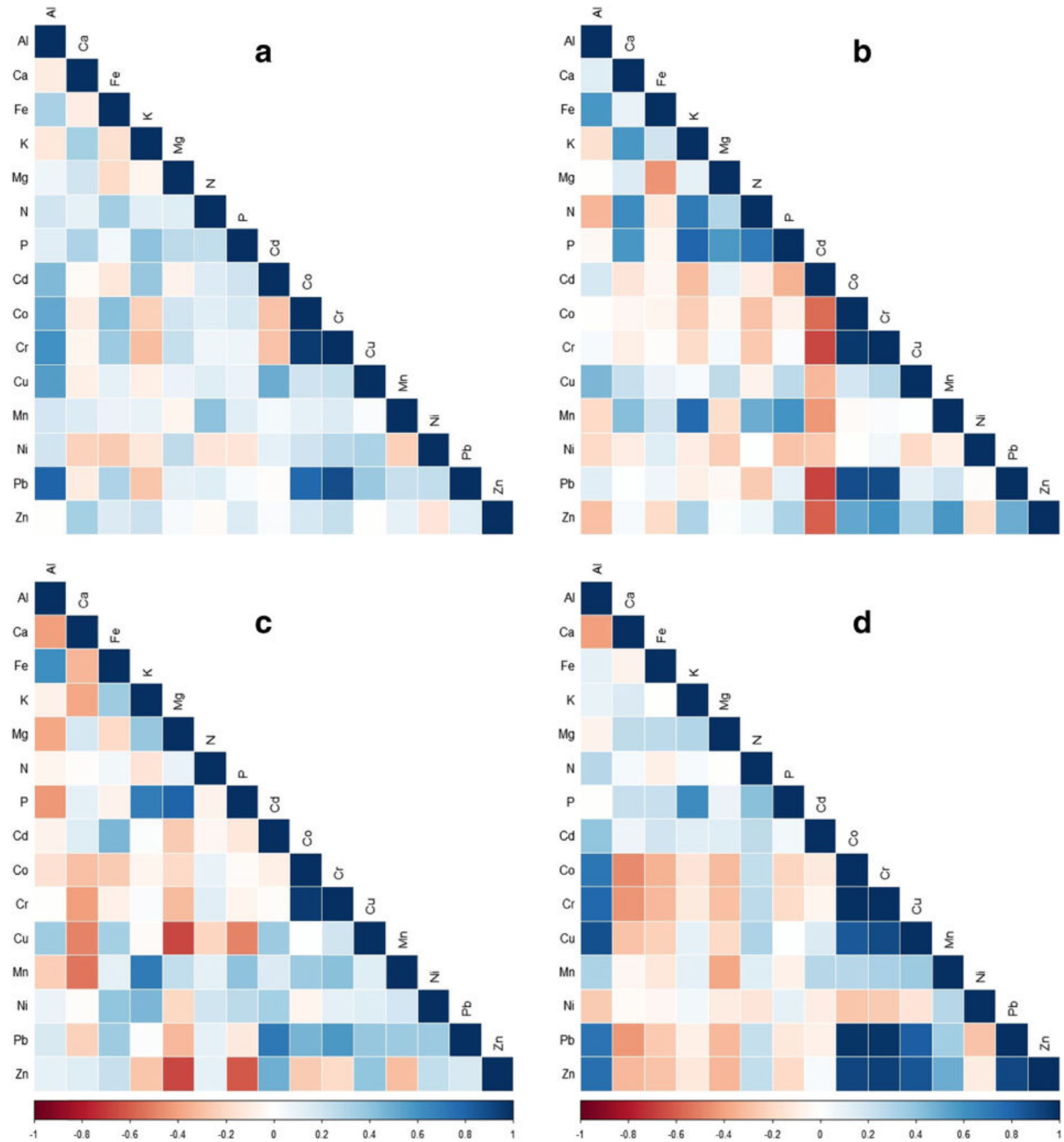

Fig. 4.

Correlation between major and trace elements concentrations in leaves at **a** Nottingham, Unionville and *S. scoparium* at Sugartown, **b** soil from *A. gerardii* and *S. nutans* at Sugartown, **c** Palmerton and **d** Fort Indiantown Gap. The three species at Sugartown were separated into two groups after our soil analyses indicated that *S. scoparium* clustered with the serpentine group while *A. gerardii* and *S. nutans* did not

Table 1

Nested analyses of variance for pseudo-total and extractible concentrations of a) major and b) trace elements in rooting soil and in leaves accounted for Site and Species (Site)

a) Source	*Df*	Al	Ca	Fe	K	Mg	Ca:Mg	N:P	P	
		F	*F*	*F*	*F*	*F*	*F*	*F*	*F*	
Pseudo-Total										
Site	*4*	10.1 ***	22.8 ***	12.1 **	29.2 ***	56.4 ***	127 ***	nd	nd	
Species (Site)	*9*	1.3 ^{ns}	2.91 *	2.47 *	2.49 *	4.71 **	2.89 ^{ns}	nd	nd	
Residuals	*89*									
Extractible										
Site	*4*	55.9 ***	20.7 ***	26.7 ***	0.537 ^{ns}	96.5 ***	193 ***	nd	28.4 ***	
Species (Site)	*9*	0.43 ^{ns}	1.19 ^{ns}	4.95 **	2.03 *	3.24 **	5.18 **	nd	4.83 **	
Residuals	*89*									
Leaves										
Site	*4*	4.3 **	72.1 ***	23.2 ***	10.6 ***	56.3 ***	144 ***	14.2 ***	18.2 ***	
Species (Site)	*9*	5.52 ***	10.8 ***	0.706 ^{ns}	0.761 ^{ns}	5.66 ***	11.2 ***	5.1 ***	8.44 ***	
Residuals	*98*									
b) Source	*Df*	Cd	Co	Cr	Cu	Mn	N	Ni	Pb	Zn
		F	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*
Pseudo-Total										
Site	*4*	930 ***	58.8 ***	102 ***	45.2 ***	16.5 **	nd	180 ***	58.8 ***	181 ***
Species (Site)	*9*	2.39 *	2.79 *	2.48 **	3.37 **	1.96 ^{ns}	nd	6.79 ***	2.12 *	2.36 *
Residuals	*89*									
Extractible										
Site	*4*	150 ***	43.1 ***	nd	3.29 *	18.4 ***	nd	91.2 ***	8.26 ***	65.2 ***
Species (Site)	*9*	3.66 **	2.1 *	nd	1.11 ^{ns}	1.64. ^{ns}	nd	4.81 ***	3.86 **	2.92 *
Residuals	*89*									
Leaves										
Site	*4*	43.7 ***	1.83 ^{ns}	3.03 ^{ns}	2.74 *	19.9 ***	1.92 ^{ns}	1.97 ^{ns}	16.3 ***	116 ***

Species (Site)	*9*	2.31 *	1.32 ns	1.75 ns	6.17 ***	1.85 ns	2.47 *	0.44 ns	2.28 *	1.24 ns
Residuals	*98*									

nd not determined. Significant level: ns $p > 0.05$;

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.001$