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Does hyperaccumulated nickel affect leaf decomposition? A field test using *Senecio coronatus* (Asteraceae) in South Africa

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Summary. Nickel hyperaccumulator plants contain unusually elevated levels of Ni (>1,000 mg Ni kg^{-1}). The high Ni concentration of hyperaccumulator tissues may affect ecosystem processes such as decomposition, but this has yet to be studied under field conditions. We used *Senecio coronatus* Thunb. (Harv.) from two pairs of serpentine sites: one member of each pair contained a hyperaccumulator population and the other a non-hyperaccumulator population. Our main goal was to determine if leaf Ni status (hyperaccumulator or non-hyperaccumulator) affected leaf decomposition rate on serpentine sites. We also used a non-serpentine site on which leaves from all four *S. coronatus* populations were placed to compare decomposition at a single location. Dried leaf fragments were put into fine-mesh (0.1 mm) nylon decomposition bags and placed on field sites in mid-summer (early February) 2000. Sets of bags were recovered after 1, 3.5, and 8 months, their contents dried and weighed, and the Ni concentration and total Ni content of high-Ni leaves was measured. For the serpentine sites, there was no significant effect of leaf Ni status or site type on decomposition rates at 1 and 3.5 months. By 8 months, leaf Ni status and site type significantly influenced decomposition on one pair of sites: hyperaccumulator leaves decomposed more slowly than non-hyperaccumulator leaves, and leaves of both types decomposed more slowly on the non-hyperaccumulator site. At the non-serpentine site, the highest-Ni leaves (15,000 mg Ni kg^{-1}) decomposed more slowly than all others, but leaves containing 9,200 mg Ni kg^{-1} did not decompose more slowly than non-hyperaccumulator leaves. Nickel in decomposing hyperaccumulator leaves was released rapidly: after 1 month 57 – 68 % of biomass was lost and only 9 – 28 % of original Ni content remained. We conclude that very high (>10,000 mg Ni kg^{-1}) leaf Ni concentrations may slow decomposition and that Ni is released at high rates that may impact co-occurring litter- and soil-dwelling organisms.

Key words. Decomposition – ecosystem processes – Ni hyperaccumulation – serpentine soil – ultramafic soil

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Introduction

Decomposition is an important ecosystem process that recycles the elements upon which life depends. Three major factors affect the decomposition rate of plant litter (Aerts 2006): 1) climate, 2) the nature of the soil organisms involved, and 3) the chemical composition of the organic matter being decomposed. Nickel hyperaccumulator plants produce chemically unusual organic matter containing extremely elevated levels of Ni (Reeves & Baker 2000). These plants typically grow on serpentine (ultramafic) soils, which also are chemically unusual because they have low Ca:Mg ratios and often elevated levels of metals such as Cr and Ni (Nagy & Proctor 1997). Nickel hyperaccumulator plants contain at least 1,000 mg Ni kg^{-1} (dry mass basis) in the aboveground parts of at least one specimen collected from the wild (Reeves 1992), but maximum levels in some species exceed 30,000 mg Ni kg^{-1} (Reeves *et al.* 1996, 1999). Compared to most serpentine soil plant species, which have Ni levels of <100 mg Ni kg^{-1} (Reeves 1992), Ni hyperaccumulator tissues contain 1 – 3 orders of magnitude more Ni and thus are chemically unusual substrates for decomposers.

Boyd and Martens (1998) suggested that the high Ni concentration of hyperaccumulator tissues may affect ecosystem processes such as detritivory and decomposition. Based upon the general toxicity of Ni (Pais & Jones 1997), it may be expected that hyperaccumulator tissues will decompose more slowly than tissues with lower Ni

concentrations. However, Boyd and Martens (1998) and Boyd (2007) pointed out that serpentine sites probably host Ni tolerant herbivores and mycorrhizal fungi that take advantage of the resource represented by hyperaccumulator plant tissues. For example, mycorrhizal associations of several Ni hyperaccumulators have recently been reported (Turnau & Mesjasz-Przybylowicz 2003, Perrier *et al.* 2006, Amir *et al.* 2007) and Amir *et al.* (2007) showed greater Ni tolerance of fungi associated with roots of strong Ni hyperaccumulators from New Caledonia. Nickel tolerant bacteria also have been reported in association with Ni hyperaccumulators (e.g., Schlegel *et al*. 1992, Idris *et al*. 2004). In a like manner, there may be Ni resistant detritivores/decomposers in serpentine habitats that specialize on hyperaccumulator tissues. Thus, it is unclear if the decomposition rate of hyperaccumulator litter on serpentine sites would be affected by its Ni concentration. High tissue Ni levels might inhibit use by some detritivores/decomposers, but Ni tolerant specialists might replace inhibited organisms and break down hyperaccumulator detritus.

While some studies have documented decomposition on serpentine soils (e.g., Franck *et al*. 1997), very few have included hyperaccumulator species. Zhang *et al.* (2005, 2007) documented a rapid release of Ni from shredded biomass of the Ni hyperaccumulator, *Alyssum murale* (Waldst. & Kit.) (Brassicaceae), added to serpentine and non-serpentine soils under laboratory conditions. Boucher *et al.* (2005) compared decomposition of high- and low-Zn leaves of the Zn hyperaccumulator *Arabidopsis halleri* (L.) (Brassicaceae) in soil microcosms containing an agricultural non-serpentine soil, finding similar rates of carbon mineralization for the easily decomposable fraction but slower rates for high-Zn leaves when only the less decomposable fraction was considered. In a laboratory detritivore study, Gonçalves *et al.* (2007) reported reduced leaf consumption and survival of *Porcellio* sp. (Isopoda: Porcellionidae) offered leaves of a Ni hyperaccumulator (*Alyssum pintodasilvae* Dudley: Brassicaceae) when compared to leaves of several non-hyperaccumulator species. Besides these initial studies, none of which examined detritivory or decomposition under field conditions, the effects of hyperaccumulated metals on decomposition are unexplored.

The high Ni concentration of hyperaccumulator tissues will cause a large release of Ni during decomposition. The intensity of this Ni flux will depend on both the level of Ni hyperaccumulation and the rapidity of Ni mineralization. We know of no field studies that have documented this flux, yet it could be an important feature of serpentine communities that contain hyperaccumulator species. If some detritivore and decomposer organisms are sensitive to Ni, its localized release from decomposing hyperaccumulator leaves might influence the abundance and activity of these organisms. Thus, the spatial distribution of Ni hyperaccumulator litter could affect the structure and function of local detritivore/decomposer community assemblages in serpentine soils.

Metal hyperaccumulation has been hypothesized to benefit hyperaccumulator plants in several ways (Boyd & Martens 1992, Boyd 2007). In elemental allelopathy (Boyd & Jaffré 2001), Ni taken up by plants from the soil profile is deposited into surface soil layers underneath the canopy of a Ni hyperaccumulator. By enriching those layers in Ni, a hyperaccumulator may create a soil microenvironment that is unsuitable for less Ni tolerant plant species. In addition, several authors (e.g., Ernst 1972, Wild 1978, Baker 1981) have suggested that shedding high-metal leaves might function as a metal disposal mechanism that allows hyperaccumulators to "detoxify" high Ni soils by removing Ni from the soluble pool in the rooting zone. Boyd and Martens (1992) pointed out that decomposition would release this metal back into surface soil once leaves decomposed (or were burned by fire) and thus concluded this benefit was unlikely. However, Boyd and Martens (1992) did not consider that high metal leaves might decompose more slowly than low metal leaves. If high metal leaves decompose more slowly, then concentrating metal in dropped leaves would cause that metal to accumulate in the litter under hyperaccumulator canopies. Over time, this might remove significant quantities of metal from the rooting zone and produce a soil detoxifying effect. As mentioned above, however, decomposition rates of metal hyperaccumulator leaves under field conditions are unexplored, so that it is unknown if this detoxifying effect may occur in the field.

Our study focused on *Senecio coronatus* (Thunb.) Harv. (Asteraceae), a Ni hyperaccumulator species in which some serpentine populations hyperaccumulate Ni whereas others do not. Morrey et al. (1992) reported leaves contained up to 24,000 mg Ni kg^{-1} whereas Boyd et al. (2002) found serpentine populations containing less than 150 mg Ni kg^{-1}. Therefore, this species is particularly useful for studying the effects of Ni concentration on decomposition because both hyperaccumulator and non-hyperaccumulator tissues can be collected from plants growing on serpentine soils in the field. Morrey et al. (1992) documented total Ni levels in Mpumalanga serpentine soils as between 2,000 and 7,000 mg Ni kg^{-1} dry soil, so that plant Ni concentrations can be more than twice as high as soil concentrations.

The major goal of this study was to determine if hyperaccumulated Ni affected leaf decomposition rates under field conditions. We hypothesized that hyperaccumulator leaves would decompose more slowly than non-hyperaccumulator leaves. We also compared decomposition rates of leaves from hyperaccumulator and non-hyperaccumulator populations at a non-serpentine soil site to determine if hyperaccumulated Ni affected decomposition in that field setting. We hypothesized that an effect of hyperaccumulated Ni would be even more apparent in a non-serpentine environment because of the relatively low Ni levels in the soils and communities typically found in those locations.

Methods

Study species: *Senecio coronatus* grows in grasslands in Southern Africa. It is an herbaceous perennial with a shortened, upright, subterranean stem that produces long (up to 20 cm), broad, simple leaves aboveground and relatively fleshy roots belowground (Hilliard 1977). *Senecio coronatus* is unusual among hyperaccumulators in that some populations on serpentine soils hyperaccumulate Ni yet others do not. Morrey *et al.* (1992) reported that *S. coronatus* specimens collected from two serpentine sites in north-eastern South Africa contained up to 24,000 mg Ni kg^{-1}, but both Morrey *et al.* (1992) and Smith *et al.* (2001) noted that the species also occurred on non-serpentine soils. Boyd *et al.* (2002) reported mean leaf Ni concentrations of 12,100 and 680 mg Ni kg^{-1} from two populations growing on serpentine soils in Mpumalanga Province, South Africa. Thus, this species occurs on serpentine soils as either hyperaccumulator or non-hyperaccumulator populations. Mesjasz-Przybylowicz *et al.* (1997) suspected these populations were genetically different because they grew in areas that hosted other Ni hyperaccumulator species and these other species consistently hyperaccumulated Ni at all sites. To our knowledge, however, soil Ni concentrations under hyperaccumulating and non-hyperaccumulating *S. coronatus* plants have not been documented. Whether this phenotypic difference is due to plant genotype, soil Ni levels, or a combination of these factors, is not known.

Study sites: The area east of Badplaas (Lat. 25° 57′ S, Long. 30° 33′ E) in Mpumalanga Province, South Africa, contains scattered outcrops of serpentine soils (Morrey *et al*. 1992, Smith *et al.* 2001). Our preliminary field exploration of these serpentine areas revealed a number of *S. coronatus* localities. Populations at some of these sites hyperaccumulated Ni, whereas others did not. Our main study sites were located on serpentine exposures with grassland vegetation typical for these areas (Smith *et al*. 2001). The four sites, used previously in a study of two Ni hyperaccumulator *Berkheya* species (Boyd *et al*. 2004), were: 1) Doyershoek, where plants were located along a firebreak on a steep hillside; 2) near a small airfield close to Doyershoek that we call here the "Airstrip Serpentine" site; 3) Groenvaly, which had plants at the foot of a serpentine hill; and 4) Groenvaly Mine, an abandoned mine site about 1 km from the Groenvaly site. All of these sites hosted the Ni hyperaccumulator serpentine endemic *Berkheya coddii* Roessler (Asteraceae) and all those populations hyperaccumulated Ni (Boyd *et al.* 2004), suggesting that soil Ni levels were not the cause of the dramatically different phenotypes of *S. coronatus* on these sites. Testing of *S. coronatus* plants in the field with dimethylglyoxime (DMG) paper (Reeves 1992) showed that the populations at Doyershoek and Groenvaly hyperaccumulated Ni whereas those at Airstrip and Groenvaly Mine did not. Therefore, we will refer to the Doyershoek and Groenvaly sites as H1 and H2 (H standing for "Hyperaccumulator"), and the Airstrip Serpentine and Groenvaly Mine sites as N1 and N2 (N standing for "Non-hyperaccumulator"). These designations also show how we paired the sites into two sets, each set consisting of a hyperaccumulator and a non-hyperaccumulator *S. coronatus* population.

An additional non-serpentine study site also was selected. This site was located on a powerline right-of-way close to the Doyershoek (H1) site. Inspection of the vegetation of this site showed that serpentine indicator species of this region, such as *Berkheya coddii* (Morrey *et al*. 1992), were lacking. Despite this difference in species composition, the physiognomy of this site was similar to the grassland vegetation found on the serpentine sites and thus made a good comparative non-serpentine study site.

Mpumalanga summers are warm and moist, due to rain and thunderstorms, whereas winters are cold and dry. Nelspruit is the closest major town to the study sites, with average daily maxima per month (Schulze 1972) ranging between 23 °C (June and July) and 29 °C (December, January and February) and minima between 7 C (June and July) and 19 °C (January and February). The lowest recorded temperature is between -2 °C (July) and 12 °C (February). Average monthly rainfall ranges between 10 mm (June) and 151 mm (December) (Schulze 1972). This highly seasonal climate leads to vigorous plant growth in the summer months, which is arrested either by low temperatures (e.g., lowest recorded temperature in April is 4 °C and in May is 2 °C) or low rainfall (e.g., rainfall declines to 53 mm in April and then 10–19 mm per month between May and August). The dead plant material provides fuel for fires, which may burn as often as annually in high elevation, high rainfall areas.

Decomposition experiments: To characterize initial leaf condition, we collected mature leaves from plants in all four populations, dried them to constant weight in a convection oven at ca. 60°C, and analyzed them for elemental composition. Plant material was not rinsed with water prior to grinding and analysis to avoid leaching elements from the tissues. Ten composite leaf samples were created for each population and finely ground using a Wiley mill. Plant material was analyzed by dry-ashing and analysis of the ash dissolved in concentrated acids. Samples were dry-ashed at 485°C, oxidized further in 1 M HNO_3, and the residues re-dissolved in 1 M HCl. Element concentrations were determined using an inductively-coupled argon plasma (ICP-AE) spectrophotometer (SPECTRO CIROS CCD: Kleve, Germany).

Leaves were cut into fragments ranging from approximately 1–4 cm^{-2} in area and oven-dried at 60°C. Fine-mesh (ca. 10 holes per mm) nylon decomposition bags (10 × 10 cm square) were filled with 2.5 g of dried leaf pieces. The small mesh size of the nylon bags prevented loss of small leaf fragments and allowed access by bacteria, fungi and small soil invertebrates, but excluded invertebrates larger than 0.1 mm in diameter. Bags containing leaves of each population from a pair of sites were placed at both sites of that pair to compare decomposition of hyperaccumulating and non-hyperaccumulating leaves at hyperaccumulator and non-hyperaccumulator site types. Decomposition bags were numbered with permanent marker and attached to one another into groups of ten along pieces of string (ca. 3 m long) stapled to each bag so that recovery would be easier. We alternated the placement of bags from hyperaccumulator and non-hyperaccumulator populations along the strings so that they would be evenly spread across each site.

We also placed bags from all four *S. coronatus* populations at the non-serpentine site to compare decomposition at a single (non-serpentine) location. As above, bags were attached into groups of ten (3 each from populations H1 and N1 and 2 each from populations H2 and N2) along pieces of string and arranged so that the bags containing leaf fragments from each source population would be spread evenly over the study area.

At both serpentine and non-serpentine sites, bags were placed on the soil surface in mid-summer (1 February 2000) and the strings anchored to the ground to keep bags in place. In an effort to protect litter bags from fire, we mowed a 1-m wide firebreak around each 4 × 10 m rectangular site. On the serpentine sites, we collected 5 bags of leaves from each paired population (ten bags total from each site) in late summer (on 5 March, after 1 month) and another ten bags from each site in late fall (on 15 May, after 3.5 months). Early the following spring (on 30 September, after 8 months), we collected all 20 remaining bags from each site of the H1/N1 site pair. Bags at the second pair of sites (H2/N2) were lost from the study because they were burned in a grass fire that occurred after 7 months. On the non-serpentine site, we collected ten bags total (3 each from populations H1 and N1 and 2 each from populations H2 and N2) on 5 March and again ten bags on 15 May, and the remaining 40 bags on 30 September.

Some bags (14 % of the 180 placed into the field) were damaged by the activity of animals, resulting in tearing of the bag surface: these were excluded from the study. After exclusion, 107 bags remained from the 120 bags collected from the serpentine sites, and 48 bags remained from the 60 collected from the non-serpentine site. Remaining bags were air-dried, opened, and the leaf pieces removed and oven-dried for 72 h at 60°C. After drying, the fragments were weighed to determine mass loss. Pieces of hyperaccumulator leaves from the 1 month and 3.5 month collections were analyzed for Ni concentration by dry-ashing and analysis of the ash re-dissolved in 1 M HCl using an atomic absorption spectrophotometer (Instrumental Laboratory, IL 251). Analysis of Ni concentrations of hyperaccumulator leaf material remaining in the bags collected at 8 months was attempted, but those samples were lost from the study due to a laboratory error.

Statistical analysis: Mass loss data (fraction of original mass in each litter bag) for each pair of serpentine sites were analyzed by 2-way

Table 1. Elemental analysis of leaf samples from the four *S. coronatus* populations used. Values are means with Standard Error (SE) in parentheses, N = 10. Superscripts denote means for an element that differ significantly among populations using Fisher's Protected Least Significant Difference (PLSD) test, $P \leq 0.05$. The Variation Among Means column describes the relative values of means for each element as the greatest mean divided by the least mean.

Element (units)	Population Pair 1		Population Pair 2		Variation Among Means (greatest mean/least mean)
	H1 (Doyershoek)	N1 (Airstrip)	H2 (Groenvaly)	N2 (Groenvaly Mine)	
Ca (g/kg)	3.6^{b} (0.11)	5.1^{a} (0.079)	2.7^{c} (0.13)	3.6^{b} (0.085)	1.4
Cu (mg kg^{-1})	9.2 (2.6)	13 (3.5)	8.8 (1.7)	12 (3.6)	1.5
Fe (mg kg^{-1})	90^{a} (15)	50^{b} (10)	21^{b} (3.3)	120^{a} (11)	5.7
K (g/kg)	1.0^{c} (0.064)	1.4^{b} (0.081)	1.9^{a} (0.054)	1.8^{a} (0.097)	1.9
Mg (g/kg)	2.7^{d} (0.061)	3.1^{c} (0.063)	3.6^{a} (0.044)	3.3^{b} (0.052)	1.3
Mn (mg kg^{-1})	65^{a} (2.3)	41^{b} (2.6)	41^{b} (2.0)	38^{b} (2.1)	1.7
P (g/kg)	$0.066^{a,b}$ (0.002)	0.058^{c} (0.001)	0.065^{b} (0.002)	0.072^{a} (0.003)	1.2
Ni (mg kg^{-1})	$15{,}000^{a}$ (610)	16^{c} (2.6)	9200^{b} (670)	130^{c} (13)	940
Zn (mg kg^{-1})	73^{a} (3.2)	14^{c} (0.066)	54^{b} (5.2)	15^{c} (0.63)	5.2

Analysis of Variance (ANOVA), using a separate ANOVA for each site pair and with site type (hyperaccumulator or non-hyperaccumulator) and leaf Ni status (hyperaccumulator or non-hyperaccumulator) as main effect factors (Abacus Concepts 1998). Non-serpentine site data were analyzed by a 1-way ANOVA to test the influence of leaf source on mass loss. In this case, we classified leaves by their source population (H1, H2, N1, N2) rather than simply as hyperaccumulator or non-hyperaccumulator. We did this because analysis of leaf samples showed that H1 and H2 leaves differed significantly in Ni concentration (see Results) and thus represented different degrees of Ni hyperaccumulation. Mass loss data were arcsin-square root transformed prior to ANOVA so they would meet the statistical assumptions underlying that analysis (Zar 1996).

We also examined changes in both Ni concentration and Ni content of hyperaccumulator leaf material. We used ANOVA to examine the effects of time (using initial data and values at 1 and 3.5 months) as well as site (hyperaccumulator vs. non-hyperaccumulator) on these parameters of hyperaccumulator leaves. Changes over time in Ni concentration and content for non-hyperaccumulator leaves were not documented due to both the relatively small amount of biomass remaining in recovered litter bags and the low Ni concentrations of those leaves, resulting in very large standard errors and data that we considered unreliable. For all ANOVAs, Fisher's Protected Least Significant Difference (PLSD) test was used for post-hoc means separations (Abacus Concepts 1998).

Results

Initial leaf analysis: Leaves from the four *S. coronatus* populations varied significantly in composition for all elements excepting Cu (Table 1). The greatest variation between populations of all elements tested was for Ni concentration, which ranged 940-fold. The Ni concentrations of leaves of the two non-hyperaccumulator populations (N1 and N2) did not differ significantly from each other, whereas the hyperaccumulator populations differed from the non-hyperaccumulators as well as from one another (Table 1). Population means for two other metals, Fe and Zn, also varied substantially: between 5- and 6-fold (Table 1). The pattern of variation for Zn matched that of Ni, with H1 having greatest levels, H2 with next highest levels, and both non-hyperaccumulator populations having similarly low concentrations. The remaining elements that varied significantly among populations (Ca, K, Mg, Mn, P) all varied relatively little (between 1.2- and 1.9-fold) and in a variety of patterns (Table 1).

Decomposition on serpentine sites: Mass loss after one month was large, ranging from 57–63 % for leaves on the first pair of sites and 63–68 % for leaves on the second pair (Fig. 1). Relatively little additional mass loss occurred at 3.5 and 8 months (Fig. 1). ANOVA of data from site pair 1 (N1/H1) showed no effect of leaf Ni status ($F_{1,16} = 0.12$, $P = 0.73$), site type ($F_{1,16} = 2.7$, $P = 0.12$) or the interaction ($F_{1,16} = 0.04$, $P = 0.84$) at 1 month. We also found no significant effects of leaf Ni status ($F_{1,13} = 2.2$, $P = 0.16$), site type ($F_{1,13} = 2.1$, $P = 0.17$) or the interaction ($F_{1,13} = 0.49$, $P = 0.50$) at 3.5 months. At 8 months, however, both leaf Ni status ($F_{1,34} = 7.2$, $P = 0.011$) and site type ($F_{1,34} = 8.6$, $P = 0.006$) significantly affected mass loss, although the interaction remained insignificant ($F_{1,34} = 0.29$, $P = 0.59$). Figure 1 shows that mass loss at 8 months was less for H1 compared to N1 leaves, and also was less on the H1 site than on the N1 site. Data from the N2/H2 site pair gave similar results for the 1 and 3.5 month time periods (Fig. 1). At 1 month there was no significant effect of leaf Ni status ($F_{1,15} = 0.91$, $P = 0.35$), site type ($F_{1,15} = 1.2$, $P = 0.29$) or the interaction ($F_{1,15} = 0.54$, $P = 0.47$). At 3.5 months, also, we found no significant effect of leaf Ni status ($F_{1,9} = 0.74$, $P = 0.41$), site type ($F_{1,9} = 0.63$, $P = 0.45$) or the interaction ($F_{1,9} = 0.56$, $P = 0.48$). There are no results from this second pair of sites at 8 months because the litter bags were destroyed by fire.

Nickel concentration of hyperaccumulator leaves decreased markedly after 1 month. H1 leaf Ni concentration decreased 40–64 %, depending on site (Fig. 2a), whereas H2 leaves decreased 63–73 % (Fig. 2b). No further decline was documented at 3.5 months. ANOVAs of leaf Ni concentration showed highly significant effects of time on Ni concentration in three of four cases: H1 leaves on the H1 site ($F_{2,15} = 26$, $P < 0.0001$), H2 leaves on the H2 site ($F_{2,17} = 79$, $P < 0.0001$), and H2 leaves on the N2 site ($F_{2,13} = 61$, $P < 0.0001$). The exception was a marginally significant result for H1 leaves on the N1 site ($F_{2,17} = 3.5$, $P = 0.053$). The Ni concentration of hyperaccumulator leaves

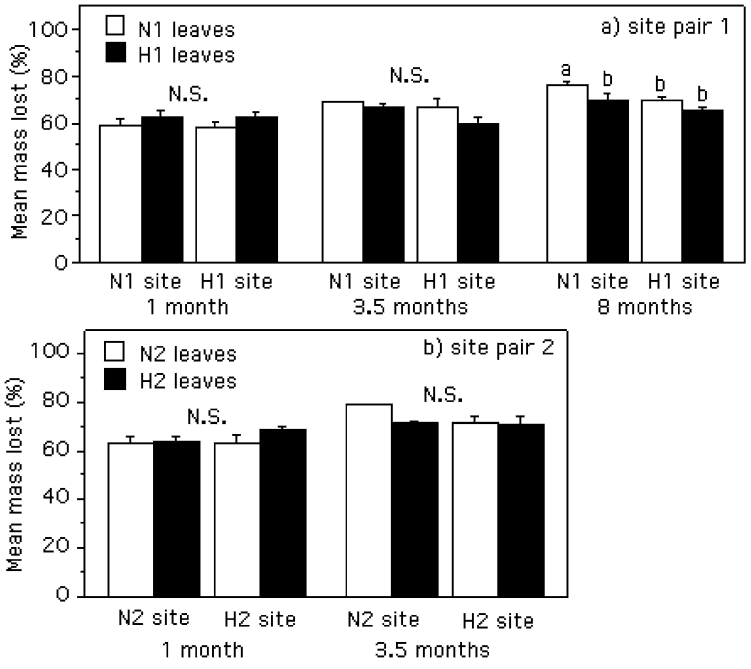

Fig. 1 Mass loss (percent of initial mass) of leaf material in litter bags placed onto (a) site pair 1 and (b) site pair 2. Data are means + SE. Means with different letters differ significantly (Fisher's PLSD test) at $P \leq 0.05$ ("N.S." signifies means were not significantly affected by site type or leaf Ni status). Site pair 2 lacks data from 8 months because litter bags on this site were destroyed after 7 months by a grassland fire

was not influenced by site type. For H1 leaves after 1 month, we found a marginally significant influence of site type on Ni concentration ($F_{1,6} = 5.7$, $P = 0.054$), whereas for H2 leaves this effect was non-significant ($F_{1,7} = 0.44$, $P = 0.53$). Site type was non-significant by 3.5 months for both H1 ($F_{1,8} = 0.29$, $P = 0.67$) and H2 leaves ($F_{1,5} = 1.1$, $P = 0.35$).

Total Ni contents of hyperaccumulator leaf bags declined greatly during the first month (Fig. 3). Initial Ni contents per bag, calculated by combining initial Ni concentrations (Table 1) with the mass of leaf fragments in each bag (N = 10 for each, SE in parentheses) were: 36,000 (1500) μg Ni for H1 leaves; 23,000 μg Ni (1700) for H2 leaves; 330 μg Ni (33) for N2 leaves; and 40 μg Ni (6.4) for N1 leaves. After 1 month, total Ni contents for hyperaccumulator leaves had declined steeply (Fig. 3a,b). Much of this decline was due to the large percentages of mass lost from the litter bags by that time (at least 57%: see Fig. 1). ANOVAs of leaf Ni content showed highly significant effects of time on Ni content for H1 leaves on the H1 site ($F_{2,15} = 100$, $P < 0.0001$), H1 leaves on the N1 site ($F_{2,17} = 78$, $P < 0.0001$), H2 leaves on the H2 site ($F_{2,17} = 66$, $P < 0.0001$), and H2 leaves on the N2 site ($F_{2,13} = 37$, $P < 0.0001$). As with the Ni concentration data (Fig. 2), Ni content of hyperaccumulator leaves after 1 month was not significantly affected by site type (site pair 1: $F_{1,6} = 3.9$, $P = 0.096$; site pair 2: $F_{1,5} = 3.9$, $P = 0.10$), nor was Ni content affected by site type after 3.5 months (site pair 1: $F_{1,8} = 0.004$, $P = 0.95$; site pair 2: $F_{1,5} = 0.67$, $P = 0.45$). Nickel contents of hyperaccumulator leaf bags were uniformly much reduced (< 30% of initial content) at both 1 and 3.5 months (Fig. 3a,b).

Decomposition on the non-serpentine site: Decomposition also was rapid on the non-serpentine site. At one month, about 63% (range: 55–68%) of leaf mass had been lost on this site (Fig. 4), which was comparable to mass losses on the serpentine sites (Fig. 1). The source population significantly affected mass loss (ANOVA: $F_{3,6} = 6.9$, $P = 0.023$). Leaves from the H1 population lost significantly less mass than those from both site 2 popu-

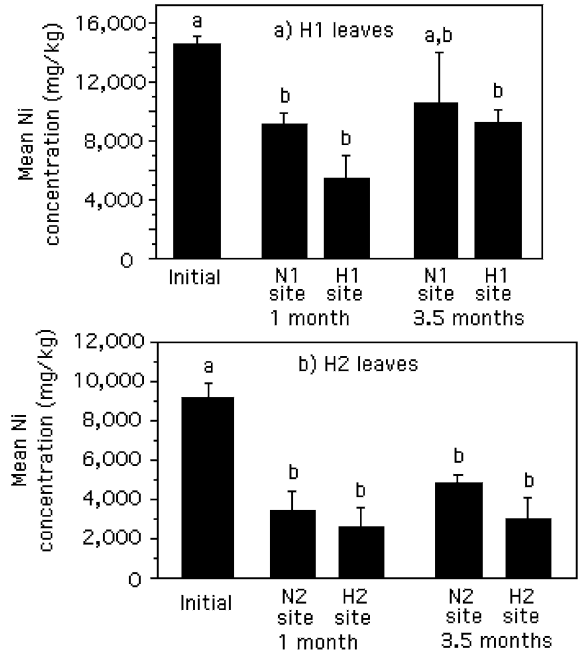

Fig. 2 Nickel concentrations of hyperaccumulating leaves (means + SE) for (a) site pair 1 and (b) site pair 2 at the start of the study (initial) and after 1 month and 3.5 months. Means with different letters within each figure panel are significantly different (Fisher's PLSD test, $P \leq 0.05$)

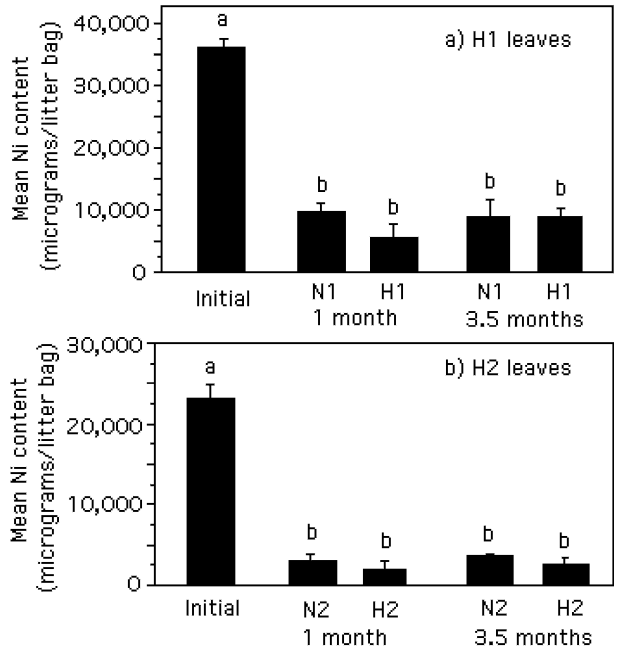

Fig. 3 Nickel contents of hyperaccumulating leaf litter bags (means + SE) for (a) site pair 1 and (b) site pair 2 at the start of the study (initial) and after 1 month and 3.5 months. Means with different letters within each figure panel are significantly different (Fisher's PLSD test, $P \leq 0.05$)

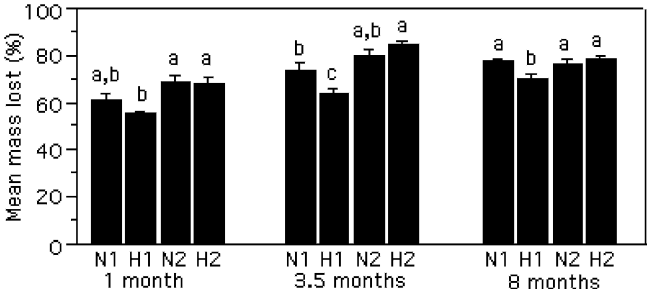

Fig. 4 Mass loss of leaf material placed onto the non-serpentine site after 1 month, 3.5 months and 8 months. Data are means (+ SE). For data within each time interval, means with different letters differ significantly (Fisher's PLSD test, $P \leq 0.05$)

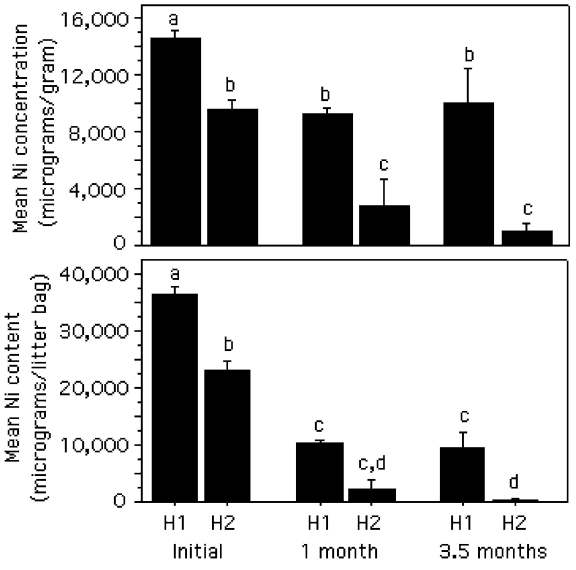

Fig. 5 Means of (a) Ni concentrations and (b) Ni contents for hyperaccumulating leaf samples placed onto the non-serpentine site at the start of the study (initial) and after 1 month and 3.5 months. Data are means (+ SE). Within each figure panel, means with different letters are significantly different (Fisher's PLSD test, $P \leq 0.05$)

lations, while the N1 leaves lost an intermediate amount (Fig. 4). Mass loss also was significantly affected by population at 3.5 months (ANOVA: $F_{3,6} = 13$, $P = 0.005$), and again H1 leaves lost significantly less mass than all others. At 8 months, population again significantly influenced mass loss (ANOVA: $F_{3,24} = 5.7$, $P = 0.004$). At this time, leaves from the H1 site once more had lost significantly less mass compared to leaves from the other three sites (Fig. 4). Although H2 leaves initially contained hyperaccumulator levels of Ni (9,200 mg Ni kg^{-1}: Table 1), their mass loss was not less than non-hyperaccumulator leaves. There was not even a trend for H2 leaves to lose less mass than either N1 or N2 leaves: indeed, at 3.5 months mass loss of H2 leaves was significantly greater than that of N1 leaves (Fig. 4).

Nickel concentrations of hyperaccumulator leaves declined rapidly. The Ni concentration of the highest Ni leaves (H1) declined about 40 % by 1 month and then remained constant at about 10,000 mg Ni kg^{-1} at 3.5 months (Fig. 5a), whereas H2 leaves declined 70 % after 1 month and continued to a 90 % decline by 3.5 months. ANOVA of the data for each leaf type showed a significant effect of time on Ni concentration (H1 leaves, $F_{2,13} = 8.6$, $P = 0.004$; H2 leaves, $F_{2,11} = 18$, $P = 0.0003$). Post-hoc tests showed significant differences between initial and later values for both H1 and H2 leaves, but not between values at 1 and 3.5 months (Fig. 5a).

Nickel content of hyperaccumulator leaves also decreased rapidly (Fig. 5b). H1 leaves lost 72 % of their Ni in the first month but little more after that (losing 74 % at 3.5 months). H2 leaves lost 90 % after 1 month and by 3.5 months had lost over 98 % of their original Ni content (Fig. 5b). ANOVA showed that time significantly affected leaf Ni content in both cases (H1 leaves, $F_{2,13} = 67$, $P < 0.0001$; H2 leaves, $F_{2,11} = 29$, $P < 0.0001$). In each case, post-hoc comparisons showed significant differences between initial and both 1 month and 3.5 month values, but no difference between 1 and 3.5 month values, indicating that the significant effect of time was mainly due to rapid Ni loss from both H1 and H2 leaves during the first month of decomposition.

Discussion

Nickel concentrations of *S. coronatus* leaves varied among populations by almost three orders of magnitude (Table 1). Most of the Ni content of hyperaccumulator

leaves was released by decomposition within the first month (Figs. 3, 5b), when summer rainfall and temperature in Mpumalanga are both relatively high (Schulze 1972). The rapid release of Ni from hyperaccumulating leaves represents a remarkable Ni flux. In the most extreme example on the serpentine sites, a decomposition bag containing leaves from the highest Ni population (H1) on the H1 site lost 30,700 (36,000 minus 5,300) μg Ni, or about 900 μg day^{-1} (assuming linear release of Ni over the 34 day period of 1 February to 5 March 2000). Most of this Ni loss was due to decomposition of biomass rather than leaching of Ni from leaf tissue. Again using the H1 bags for example, 76 % of the 30,700 μg Ni lost was due to reduction in biomass and only 24 % due to leaching of Ni from the biomass remaining in the bags after 1 month. This large flux of Ni could affect other organisms in the litter or soil, depending on the Ni sensitivity of the organism and the dose of Ni experienced. Considering that these Mpumalanga serpentine soils have total Ni values between 2,000 and 7,000 μg Ni g^{-1} dry soil (Morrey et al. 1992), the flux of Ni from decomposing *S. coronatus* leaves may impact litter and soil communities on these sites.

There was little evidence that site type (hyperaccumulator vs. non-hyperaccumulator) influenced decomposition rates on our serpentine study sites. At 1 and 3.5 months there was no influence of site type on decomposition for either site pair (Fig. 1), but by 8 months we found a significant site effect for pair 1. In that case, decomposition was less on the hyperaccumulator site. Unfortunately, loss of site pair 2 from the study left us with no replication of these 8 month results. Our conclusion overall is that decomposition rates differed little for *S. coronatus* leaves on hyperaccumulator and non-hyperaccumulator sites.

One of our major goals was to compare decomposition rates between leaves containing hyperaccumulator and non-hyperaccumulator levels of Ni. On serpentine sites, we did find significantly slower decomposition of hyperaccumulator leaves, but only at 8 months and only for one pair of sites (the one not impacted by the grassland fire). However, we also found slower decomposition for the leaves highest in Ni concentration on the non-serpentine site at 3.5 and 8 months (Fig. 4), which is consistent with the serpentine site results. This suggests that leaves with very high Ni concentrations decompose more slowly than those with lower concentrations, but this "very high" level might be $>$ 10,000 mg Ni kg^{-1}. This high threshold is suggested by results from the non-serpentine site, where H2 leaves (initial Ni concentration 9,200 mg Ni kg^{-1}) did not differ in decomposition rate from N1 or N2 leaves (initial Ni concentrations $\leq$ 130 mg Ni kg^{-1}) at 1 and 8 months and decomposed more rapidly than N1 leaves at 3.5 months (Fig. 4).

Overall, our results are consistent with those of Boucher *et al.* (2005), who compared decomposition of high- and low-Zn *Arabidopsis halleri* biomass in soil microcosms containing a non-serpentine soil. They found similar rates of decomposition overall but, when they examined the decomposition of the less decomposable fraction of carbon (mainly cell wall materials), they detected slower decomposition of high-Zn leaves. Our finding of slower decomposition of Ni hyperaccumulator leaves by 8 months, but not at 1 or 3.5 months, suggests that more easily decomposed materials were mineralized at similar rates in our leaves as well. Differential decomposition occurred when only the more resistant materials remained. We also note that the Zn concentrations of *S. coronatus* leaves differed significantly among populations (Table 1), with highest levels in the highest-Ni population (H1). This population's leaves were the ones to decompose most slowly in the field. We suggest this slowed decomposition was more likely due to the Ni level rather than the Zn level of these leaves, as the levels of Zn that affected decomposition reported by Boucher *et al.* (2005) were up to 20,500 mg Zn kg^{-1}: much higher than the 73 mg kg^{-1} we documented in our H1 leaves.

One way by which Ni released during decomposition might affect other plant species is through elemental allelopathy (Boyd & Martens 1998). Zhang *et al.* (2005, 2007) pointed out that elemental allelopathy is contingent upon the fate of Ni added to the soil by decomposition: Ni that becomes unavailable by being tightly bound to soil constituents will not contribute to elemental allelopathy. Zhang *et al.* (2007) found no evidence of elemental allelopathy in a pot study in which Ni hyperaccumulator biomass was added to soils to determine its effects on seed germination of eight herbaceous species. To our knowledge, elemental allelopathy remains untested under field conditions, although Boyd & Jaffré (2001) reported that surface soil Ni concentrations were significantly elevated under the New Caledonian Ni hyperaccumulator tree *Sebertia acuminata* Pierre *ex* Baillon (Sapotaceae), thus providing a pre-condition for elemental allelopathy. In the case of *S. coronatus*, if dead leaves remain in the vicinity of plants, then considerable Ni is released into surface soil layers during decomposition. This release would be even more rapid if leaves were burned during grassland fires, which are frequent in this region toward the end of the growing season. Future studies of *S. coronatus* might compare surface soil Ni levels around plants in hyperaccumulator and non-hyperaccumulator populations to determine if hyperaccumulator plants influence the spatial pattern of Ni concentration in surface soil. If so, then *S. coronatus* could be used to test the elemental allelopathy hypothesis under field conditions.

Our results also bear upon the hypothesis that Ni hyperaccumulation may "detoxify" high Ni soils through accumulation of undecomposed high-Ni litter (Ernst 1972, Wild 1978, Baker 1981). Although we showed that very high Ni concentrations slowed leaf decomposition, this effect was relatively weak. Most of the Ni in hyperaccumulator *S. coronatus* leaves was released quickly (Fig. 3), resulting in a large Ni flux. However, whether or not the released Ni rejoined the plant-available pool or was bound by other litter or into surface soil layers is

unknown. As mentioned above, the pot studies of Zhang *et al.* (2005, 2007) found that Ni released during decomposition of hyperaccumulator leaves was quickly bound in the soil. Further investigation of the fate of the Ni released during decomposition is warranted, but at this point hyperaccumulation seems unlikely to benefit plants via metal disposal or soil detoxification because release of Ni from hyperaccumulator litter is relatively rapid.

Our study is the first exploration of the effects of a hyperaccumulated element on decomposition under field conditions. Our experiment was able to take advantage of an unusual feature of *S. coronatus:* the existence of both hyperaccumulator and non-hyperaccumulator populations on serpentine soils. Additional experiments using other hyperaccumulator species are needed to determine if hyperaccumulator plant tissue generally decomposes more slowly under field conditions and if the threshold for a significant Ni effect is as high as in our experiment. Experiments using other hyperaccumulators probably will be unable to use field-collected tissues, as most hyperaccumulator species do not display such extreme within-species variation in element concentration (Reeves & Baker 2000, Reeves 2003). However, hyperaccumulator and non-hyperaccumulator tissues can be produced by growing plants on soils that are either high or low in metal, making hyperaccumulators model systems for conducting several types of ecological experiments (Pollard 2000). We hope future experiments will take advantage of this feature of hyperaccumulators to explore the consequences of hyperaccumulation for decomposition, as well as other ecosystem processes.

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