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Soil Biology & Biochemistry 40 (2008) 845–848

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Short Communication

Seasonal patterns of cadmium accumulation in *Arrhenatherum elatius* (*Poaceae*): Influence of mycorrhizal and endophytic fungal colonisation

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Received 1 March 2007; received in revised form 17 September 2007; accepted 20 September 2007
Available online 16 October 2007

Abstract

We investigated the influence of arbuscular mycorrhizae (AM) and dark septate fungi (DSF) colonisation on cadmium (Cd) accumulation in *Arrhenatherum elatius* from heavy metal-contaminated sites. AM colonisation disappeared when Cd concentrations in soil increased, while DSF infection was weak but constant throughout the experiment indicating that soil heavy metals are toxic to AM but not to DSF. AM colonisation was greatest when plant Cd concentrations were highest providing evidence that AM colonisation may influence Cd accumulation. In addition, the disappearance of AM and the concomitant reduction of Cd in shoots during seed maturation result in our suggestion that seasonal variation in AM may play a role in protecting developing seeds from soil pollution.
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Keywords: *Arrhenatherum elatius*; Arbuscular mycorrhizae; Cadmium; Dark septate fungi; Fungal colonisation; Seasonal variations

Cadmium (Cd) concentrations, as well as uptake and accumulation by higher plants fluctuate through the year with a significant decrease often seen during spring (Matthews and Thornton, 1982; Brekken and Steinnes, 2004). Deram et al. (2006) observed a seasonal variation of Cd in metallicolous populations of *Arrhenatherum elatius*, a perennial grass with high biomass production. Cd shoot concentrations were highest at the end of winter, decreasing until late spring and increasing again in July. This phenomenon is generally referred to as a "dilution effect" due to growth increase (Rains, 1971; Brekken and Steinnes, 2004, for a review; Jiang et al., 2004). It has also been demonstrated that the dilution effect is accompanied by a reduction in the translocation factor restricting transport of toxic elements from roots to shoot biomass, a coincidence often cited as a strategy for heavy metal tolerance (Dahmani-Muller et al., 2000; Brekken and Steinnes, 2004).

In many terrestrial ecosystems, arbuscular mycorrhizae (AM) and dark septate fungi (DSF) colonisation also vary according to seasonal patterns (Lingfei et al., 2005) and the facilitating role of mycorrhizae in tolerance and metal root uptake has been hypothesized by several workers (e.g. Citterio et al., 2005). *A. elatius* is characterised by significant root fungal colonisation by AM and DSF (Vandenkoornhuyse et al., 2002) which has led to speculation that root fungal endophytes could influence the seasonality of metal accumulation. To date, no study has examined whether the seasonality of plant metal accumulation is linked to the seasonality of root fungal colonisation; our aim was to address this question.

The two study sites, Auby and Mortagne-du-Nord are old slag heaps resulting from the processing of zinc ores.

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0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2007.09.023

These sites are contaminated with Cd, zinc (Zn) and lead (Pb). We focused on Cd since *A. elatius* accumulates relatively small amounts of Zn and Pb (Schwartz et al., 2001), but is considered a good accumulator of Cd (Deram et al., 2007).

From January to July, six samples of *A. elatius* were collected from both Auby and Mortagne-du-Nord. Samples were taken at least 30 m apart from areas dominated by *A. elatius* and where Cd and Zn concentrations in soils represented natural variability. Each sample was 15×15 cm, 30 cm deep and separated into three parts: soil was collected by shaking; roots were separated from soil; shoots were cut 5 cm above the soil level.

Soil samples (both total- and NH_4OAc/EDTA-extractable fractions), roots and shoots were analysed using methods described in Deram et al. (2006). Sub-samples of 1 cm long, fine-branched roots were taken randomly, cleared and stained with acid glycerol trypan blue (Koske and Gemma, 1989) to assess fungal colonisation. Thirty stained root segments per plant were mounted on slides in Polyvinylalcohol–Lacto–Glycerol (Koske and Tessier, 1983) and examined under an optical microscope ($\times 100$); 180 sub-samples per month, per site were analysed in this way ($n = 6 \times 30$ root sub-samples).

Frequencies of colonisation were calculated (i.e. the number of colonised roots versus the total number of observed roots) including frequency of colonisation by mycorrhizae (F_{MYC}), arbuscules (F_A), vesicles (F_V), and dark septate fungi (F_{DSF}). Proportions of the root cortex colonised by mycorrhizal and septate fungi (P_{MYC} and P_{DSF}) and arbuscules (P_A) and vesicles (P_V) occurring in colonised parts, were estimated by rating the density of infection using a five class system according to Trouvelot et al. (1985). Means of all data were compared using Students' *t*-tests (Statistica 5.1).

Variation of Cd concentrations and fungi parameters in *A. elatius* are summarized in Table 1. NH_4OAc/EDTA-extractable Cd concentrations in soil varied between sites ($p < 0.001$) and sampling times ($p < 0.001$) with highest values in May. Root Cd concentrations varied significantly between sites ($p < 0.001$) but not sampling times ($p > 0.05$). Significant seasonal variations of shoot Cd concentrations were observed ($p < 0.001$) at both sites. Shoot concentrations had maxima at the end of winter, decreased until late spring, and finally increased again in July.

A. elatius roots were colonised by both AM and DSF, which although present in the same root fragment, were not present in the same cell. Frequencies of AM (F_{MYC}) colonisation were high but proportion (P_{MYC}) were weak: no significant differences between these parameters versus sampling time were observed at either Auby or Mortagne-du-Nord, but both were nevertheless highest in early spring and lowest in summer. Frequencies and proportion of arbuscules (F_A, P_A) and vesicles (F_V, P_V) were weak and decreased from winter maxima to disappear in June and July: there were significant statistical differences

Table 1
Seasonal variation of cadmium contents (mg kg^{-1}) and mycorrhizal parameters (%) in *Arrhenatherum elatius* at Auby and Mortagne-du-Nord.

		January	March	April	May	June	July
Auby	$Soil_{NH_4Ac/EDTA}$(***)	14.80±9.52	59.10±64.84	56.21±27.72	201.07±51.31	200.00±66.01	145.80±33.56
	Root	33.94±7.66	41.06±15.94	42.06±14.10	34.64±20.32	33.55±8.16	34.33±17.24
	Shoot(***)	22.59±6.22	13.10±5.80	2.47±3.61	2.22±4.12	0.01±0.32	2.13±0.41
	F_{MYC}	47.81±26.84	68.69±30.17	59.24±35.78	50.00±38.99	32.53±35.12	34.64±18.35
	P_{MYC}	2.50±4.13	5.12±8.62	3.41±13.57	8.45±12.57	0.61±2.00	0.57±1.49
	F_A(*)	33.71±19.21	2.89±6.11	0.89±2.17	0.00±0.00	0.00±0.00	0.00±0.00
	P_A	0.88±0.98	0.65±1.45	0.25±0.61	0.00±0.00	0.00±0.00	0.00±0.00
	F_V	8.55±17.25	22.61±24.80	15.91±30.54	22.32±38.87	0.00±0.00	0.00±0.00
	P_V	0.95±2.12	3.31±4.79	4.50±8.61	5.25±8.78	0.00±0.00	0.00±0.00
	P_{DSF}	7.25±9.48	8.40±6.01	6.88±9.65	5.32±6.45	9.36±14.50	3.42±4.67
Mortagne-du-Nord	$Soil_{NH_4Ac/EDTA}$(***)	3.40±6.26	14.15±3.27	15.60±7.14	46.80±29.63	61.00±28.40	52.00±15.21
	Root	28.56±23.57	19.82±8.61	20.17±9.40	20.86±5.56	16.21±8.56	23.90±8.45
	Shoot(***)	13.32±8.57	5.31±6.62	4.15±1.13	0.81±0.44	0.03±0.18	0.68±0.96
	F_{MYC}	75.04±24.29	80.02±24.49	66.66±32.04	63.28±28.75	67.55±39.47	51.68±27.87
	P_{MYC}	14.93±13.82	20.80±12.87	10.57±12.33	6.57±10.14	9.70±8.84	4.65±5.70
	F_A	23.19±36.76	10.46±15.75	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	P_A	6.56±10.91	1.90±2.61	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	F_V(**)	32.02±33.46	19.90±14.22	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	P_V(*)	7.87±11.18	4.47±2.59	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	P_{DSF}	13.56±10.23	20.73±14.27	15.00±7.68	8.75±6.15	12.27±2.35	8.33±7.80

(Geometric mean ± standard deviation; F_{MYC}—frequency of mycorrhizal colonisation; P_{MYC}—proportions of root cortex colonised by mycorrhizae; P_A—proportions of the root cortex colonised by arbuscules; F_A—frequency of arbuscules; P_V—proportions of the root cortex colonised by vesicles; F_V—frequency of vesicles; P_{DSF}—proportions of the root cortex colonised by dark septate fungi; $Soil_{NH_4Ac/EDTA}$—NH_4Ac/EDTA extractable Cd in soil; Root—Cd in roots; Shoot-Cd in shoots).
Significant variation versus month are highlighted; with * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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