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Effects of CO_2 enrichment on plant-soil relationships of *Lepidium latifolium*

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Abstract

The exotic crucifer *Lepidium latifolium* L. (perennial pepperweed) is invading wetland and riparian habitats throughout the western United States. Based on previous field studies, our working hypothesis proposed that *L. latifolium* elevates soil nutrient acquisition ability in response to CO_2 enrichment. Replicates of *L. latifolium* were grown in a high fertility and low fertility soil (along with unplanted controls) in a glasshouse at ambient and elevated CO_2 concentrations (360 and 699 μmol mol^{-1}, respectively). Plants were harvested after 81 days and numerous plant and soil attributes measured. Above-ground plant mass was influenced by a significant CO_2 treatment $\times$ soil interaction ($P < 0.001$) with CO_2 enrichment inducing a greater proportional increase in mass for the low fertility soil. Root concentrations of citrate, malate, and ortho-phosphate and enzyme activities of amidase and asparaginase did not differ between the CO_2 treatments across soils. Above-ground tissue concentrations of N, S, P, Mg, K, Fe, and Zn consistently decreased for both soils with CO_2 enrichment, corresponding with higher biomass per unit nutrient. Plants grown in the low fertility soil had higher concentrations of N, S, P, Ca, and Mg in above-ground tissue than plants grown in the high fertility soil. Carbon dioxide enrichment decreased tissue N:S ratios by $> 20\%$ and increased, though not significant, tissue C:N ratio by 38% in high fertility soil and by 51% in low fertility soil. For most soil attributes measured, there was a main effect or interaction with soil fertility level. Soil attributes differed between soil fertility levels and, with the exception of SO_4^{2-}, were not influenced by the presence of *L. latifolium*. Soil attributes increased by CO_2 enrichment included acetate extractable Mg^{2+} (high fertility soil only), net 30 day N mineralization potential (unplanted control soils only), available N (high fertility soil), bicarbonate extractable P, soil-solution SO_4^{2-} (*L. latifolium* planted pots only), and soil-solution Mg^{2+} (high fertility control soil only). Collectively, these data tangentially support our working hypothesis that CO_2 enrichment increases nutrient availability. That availability of some nutrients increases without plant growth (control soils), however, suggests an interaction of elevated CO_2 with soil microflora.

Introduction

The exotic crucifer *Lepidium latifolium* L. (perennial pepperweed), a native of southeastern Europe and Asia, has widely invaded wetland and riparian habitats throughout the western United States (Young et al., 1995). These C_3 plants are clonal and have extensive underground, budding rootstocks that radiate in all directions from newly established plants. In 2 seasons, a single established plant becomes a small population that can be several meters in diameter. In as few as 5 years, infestations can be near monospecific with stem densities approaching 150 m^{-2} (Blank, 2002).

One potential explanation for the rapid invasion by *L. latifolium* is that the plant 'engineers' the soil to

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favor its own invasiveness (Jones et al., 1994). Previous field research demonstrated greater soil enzyme activity of several amidohydrolases in soils invaded by *L. latifolium* than in non-invaded soil (Blank, 2002). Moreover, amidohydrolase activities were significantly related to the KCl extractable N pool which suggests that *L. latifolium* had enhanced N availability. In that same study, roots of *L. latifolium* contained high levels of citrate and malate acids, which if exuded into the soil, may increase P availability (Hoffland et al., 1992). *L. latifolium* also increases Ca^{2+} concentration in the soil solution, relative to non-invaded areas (Blank and Young, 2002).

Many invasive species capitalize on various elements of global change to become more successful (Dukes and Mooney, 1999). Mechanisms by which these invasive plants increase at the expense of existing plant species are poorly understood to date, but competitive interactions among species, which may be altered by CO_2 enrichment, are likely involved (Bazzaz and Garbutt, 1988; Derner et al., 2002; Marks and Strain, 1989; Ziska, 2001). Our construct visualizes increasing concentration of atmospheric carbon dioxide as currency, which the plant can spend in innumerable ways, predicated on genetics, positive and negative feedback loops, and internal and external stimuli. For example, insect herbivory on a plant leaf is an external stimuli which may cause a plant to increase production of secondary compounds to deter herbivory (Bazin et al., 2002). Another example is the finding that *Lupinus albus* exposed to elevated CO_2 allocates more C to the production of proteoid roots which enhances greater P uptake (Campbell and Sage, 2002). We suggest that anthropogenic increases in atmospheric CO_2 have augmented the competitiveness of *L. latifolium*, and concomitantly its invasiveness, by improving plant-induced soil availability of N, P and Ca. We hypothesize that CO_2 enrichment 1) increases soil or plant root amidohydrolase and phosphatase activities (change in N or P availability via mineralization of organic matter), 2) increases citrate and malate in roots (change in soil P availability via root exudation), 3) increases pools of available soil Ca, and 4) alters plant-soil relationships of *L. latifolium*.

Materials and methods

Hypothesis testing took place in four glasshouses, two ambient and two elevated at the USDA-ARS Temple, TX (31°05′ N, 97°20′ W) research facility. The CO_2 concentration of air in each bay was measured at 4-min intervals with a model LI-6262 infrared gas analyzer (Li-Cor, Inc., Lincoln, NE, USA). The CO_2 readings were corrected for atmospheric pressure measured with a model DPI 260 pressure indicator (Druck, Inc., New Fairfield, CT, USA). The infrared analyzer was calibrated daily against four CO_2 gas standards and monthly against a LI-610 dewpoint generator (Li-Cor, Inc., Lincoln, NE, USA). Air temperature, manually set at 25 °C, was measured in the center of each bay with fine-wire (25-μm diameter) thermocouples. Pure CO_2 gas was injected into the appropriate bays as required to maintain the elevated CO_2 concentration. The CO_2 concentration of air in the ambient and elevated CO_2 treatments averaged 360 and 699 (μmol mol^{-1}), respectively. Photosynthetic photon flux density (PPFD) was measured on the glasshouse roof with a LI-190SB point quantum sensor (Li-Cor, Inc., Lincoln, NE, USA) and within the bays with 1-m long, LI-191SA, line quantum sensors (Li-Cor, Inc., Lincoln, NE, USA) mounted about plant height. On average, the daily integral of PPFD inside the bays was 70% of that measured above the glasshouse.

Two soil types were used: a high fertility substrate from the surface horizon of the Houston black series, a fine, smectitic, thermic Udic Haplustert, and a low fertility substrate from the surface horizon of the Pedernales series, a fine, mixed, thermic typic Palesustalf (Table 1). Although these soils are not present in areas where *L. latifolium* is invading, they do represent the range of soil fertility levels being invaded. Pots were filled to similar volumes with either 11.5 kg of the high fertility soil or 16 kg of the low fertility soil (different weights are because of bulk density differences of soils). The experiment was begun on 18 March, 2001 by planting 3 fresh root cuttings less than 5 cm in length of *L. latifolium* in 6 replicate pots × 2 soil types × 2 CO_2 treatments × 2 bays per CO_2 treatment = 48 total. We also filled pots with 3 replicates of unplanted control × 2 soil types × 2 CO_2 treatments × 2 bays per CO_2 treatment = 24 total. A plastic barrier plate with a 3 cm rim was placed beneath each pot to prevent leaching. Pots were watered daily with deionzied water, but never to the extent that the barrier plates overflowed. After one root section sprouted in each pot, the other 2 sections were removed. Plants were harvested on 11 June, 2001, 81 days post-emergence. For each plant, leaves were removed at the petiole and leaf area quantified with a commercial leaf scanner (LI-3000A, Li-Cor, Inc. Lincoln, NE, USA). Root systems were cleansed of adhering soil by water and

Table 1. Selected initial soil attributes of the two soils[a]

Attribute	High fertility soil	Low fertility soil
Texture	Clay	Fine sandy loam
KCl N (mmol kg^{-1})	1.06(0.33)	0.14(0.03)
Phosphatase (μmol g^{-1} hr^{-1})	3.30(0.15)	0.27(0.04)
Glutaminase (μmol g^{-1} hr^{-1})	18.9(1.8)	0
Asparaginase (μmol g^{-1} hr^{-1})	2.86(0.32)	0.06(0.02)
Amidase (μmol g^{-1} hr^{-1})	12.32(1.52)	0.44(0.04)
Urease (μmol g^{-1} hr^{-1})	4.56(0.79)	0.34(0.22)
$CaCl_2$ pH	7.49(0.03)	7.56(0.11)
Bicarbonate-extractable P (mmol kg^{-1})	0.17(0.03)	0.10(0.03)
Soil-solution Ca^{2+} (mmol L^{-1})	2.41(0.34)	1.19(0.22)
Soil-solution K^+ (mmol L^{-1})	0.10(0.01)	0.15(0.05)
Soil-solution SO_4^{2-} (mmol L^{-1})	0.19(0.03)	0.20(0.12)
Extractable K^+ (mmol kg^{-1})	0.60(0.04)	0.26(0.03)
Extractable Ca^{2+} (mmol kg^{-1})	18.02(1.40)	10.47(0.46)
Extractable Mg^{2+} (mmol kg^{-1})	0.53(0.04)	0.50(0.13)

[a]Data of average of 6 subsamples of stock soil. Standard deviations in parentheses. See methods section for references on procedures.

Table 2. Plant growth responses to atmospheric carbon dioxide enrichment for high and low fertility soils[a]

	High fertility soil			Low fertility soil		
Attribute	Ambient	Elevated	% change	Ambient	Elevated	% change
Above-ground mass (g)	7.31(1.31)	9.80(1.21)	+34	0.78(0.25)	1.22(0.50)	+56
Leaf area (cm^2)	735(137)	740(134)	+0.7	79(24)	85(28)	+8
Number of leaves	13.2(2.9)	13.1(2.6)	−0.7	5.3(1.9)	6.0(1.8)	+13

	ANOVA probability values		
	Above-ground mass	Leaf area	# leaves
CO_2	<0.001	0.830	0.805
Soil	<0.001	<0.001	0.010
CO_2 × Soil	0.001	0.979	0.641

[a]Standard deviations in parentheses. Above-ground mass measured after drying at 60 °C for 48 h.

bagged along with measured leaves. Soil in individual pots was homogenized and a subsample reserved. All samples were shipped overnight from Texas on dry ice to the Reno, NV USDA-Agricultural Research Service soil and plant analysis laboratory and kept in a 4 °C refrigerator until processed ($\leq$ 14 days).

Above-ground tissue was dried at 60 °C for 48 h, weighed and ground in a commercial mill. Total C, N and S were quantified on subsamples with a CHNS analyzer. Another subsample was ashed at 500 °C, solubilized in 1N HCl and analyzed for P (molybdenum blue chemistry), Ca, Mg, Fe, Mn, Cu, Zn (atomic adsorption spectroscopy), and Na and K (atomic emission spectroscopy) (Kalra, 1998). Roots were frozen, then a known weight was blended with a known weight of deionized ice for 2 min. A portion of the slurry was analyzed for amidase, urease, asparaginase, and glutaminase enzyme activities (Tabatabai, 1994). Another portion of the root slurry was filtered and the filtrate was analyzed for ortho-P, citrate, and malate by ion chromatography. Soil was analyzed on a fresh weight basis and recalculated to 105 °C weight on a separate subsample. Enzyme activities of acid phosphatase, amidase, urease, asparaginase, and glutaminase were measured using standard methods (Tabatabai, 1994). Available N was extracted with 2 N KCl (Bundy and Meisinger, 1994). A 30 day aerobic incubation procedure was used as a proxy for N min-

Table 3. Above-ground tissue nutrient concentration and selected tissue elemental mole ratios in response to atmospheric CO_2 enrichment by soil fertility level[a]

Attribute	High fertility soil			Low fertility soil		
	Ambient	Elevated	% Change	Ambient	Elevated	% Change
C (mmol g^{-1})	33.4(0.4)	33.3(0.8)	−0.3	30.8(1.5)	31.9(1.6)	+3.6
N (mmol g^{-1})	1.04(0.20)	0.74(0.08)	−28.8	1.41(0.32)	0.99(0.25)	−29.8
S (μmol g^{-1})	50.3(6.0)	45.7(3.3)	−9.1	91.8(16.4)	79.5(17.2)	−13.4
P (μmol g^{-1})	33.8(4.2)	27.5(8.6)	−18.6	66.6(20.2)	55.1(25.5)	−17.3
Ca (mmol g^{-1})	0.72(0.12)	0.58(0.11)	−19.4	0.96(0.20)	0.92(0.20)	−4.2
Mg (μmol g^{-1})	125(14)	101(11)	−19.2	181(35)	145(20)	−19.9
K (μmol g^{-1})	61(7)	48(3)	−21.3	56(10)	49(13)	−12.5
Fe (μmol g^{-1})	3.7(1.7)	3.2(0.9)	−13.5	8.9(5.0)	5.1(1.7)	−42.7
Mn (μmol g^{-1})	2.13(0.35)	2.06(0.42)	−3.3	2.290.39)	2.02(0.36)	−11.8
C:N	32.9(4.9)	45.4(5.8)	+38.3	22.9(5.6)	34.6(10.2)	+51.1
N:S	20.8(3.4)	16.4(2.3)	−21.2	15.9(4.6)	12.6(2.3)	−20.8

ANOVA probability values

	C	N	S	P	Ca	Mg	K	Fe	Mn	C:N	N:S
CO_2	0.694	0.161	0.367	0.598	0.261	0.055	0.233	0.480	0.344	0.208	0.039
Soil	0.085	0.085	0.029	0.061	0.011	<0.001	0.750	0.190	0.570	0.047	0.022
CO_2 × Soil	0.456	0.658	0.648	0.765	0.506	0.367	0.645	0.458	0.355	0.797	0.604

[a]Standard deviation in parentheses.

eralization potential (Hart et al., 1994) with net N mineralization determined by subtracting KCl-extractable N. Quantification of NO_3^- and NO_2^- used ion chromatography after removal of Cl^- with a colloidal silver filter. Quantification of NH_4^+ used a membrane diffusion colorimetric procedure. Cations were extracted by ammonium acetate (Thomas, 1982) with Ca^{2+} and Mg^{2+} quantified by atomic adsorption spectroscopy and Na^+ and K^+ quantified by atomic emission spectroscopy. Cations and anions in the soil-solution were extracted using immiscible displacement with CCl_4 (Mubarek and Olson, 1976). Anions in the soil-solution were quantified by ion chromatography and atomic adsorption/emission spectroscopy was used to quantify Ca^{2+}, Mg^{2+}, Na^+, and K^+. We measured the bicarbonate-extractable pool of available P (Olsen and Sommers, 1982). For all analytical measurements only certified standards were used which were made up in the same matrixes of the various extraction methods.

Data were analyzed using a mixed model split-plot ANOVA (SAS, 1999), with glasshouse bay (2 bays per CO_2 treatment) as the blocking factor. The experimental design was 2 replications of 6 (plant treatments) and 3 (controls) subsamples for each soil fertility level and CO_2 level. Analysis of plant attributes used categorical variables **CO_2** (ambient and elevated) and **Soil** (high fertility and low fertility soils) with glasshouse bay(CO_2) the error term for CO_2 and soil × glasshouse bay(CO_2) the error term for soil and the **CO_2 × Soil** interaction. Analysis of soil attributes used categorical variables **CO_2**, **Soil**, and **Plant** (planted with *L. latifolium* or unplanted controls) with glasshouse bay(CO_2) the error term for CO_2 and soil × plant × bay(CO_2) the error term for soil, plant and the soil × plant interaction. Confidence intervals at the 90% level were generated to compare means.

Results

Plant attributes

Above-ground biomass of *L. latifolium* was influenced by a significant ($P < 0.001$) CO_2 × soil interaction (Table 2). Biomass increased with CO_2 enrichment by 34% in the high fertility soil and by 56% in the low fertility soil. Growth in the high fertility soil produced significantly ($P \leq 0.01$) greater number of leaves and nearly 10 times greater leaf area than growth in the low fertility soil, but neither differed between CO_2 treatments.

Overall, above-ground tissue elemental concentration declined in the elevated CO_2 treatment; however, only Mg was significant ($P = 0.055$) (Table 3). Soil

Table 4. Biomass produced per unit nutrient in response to atmospheric CO_2 treatments for high and low fertility soils[a]

	High fertility soil			Low fertility soil		
Nutrient	Ambient	Elevated	% Increase	Ambient	Elevated	% Increase
C	2.49(0.03)	2.50(0.06)	0.4	2.71(0.13)	2.61(0.13)	3.7
N	69.7(10.4)	97.1(10.7)	39.3	52.8(11.4)	76.5(19.7)	44.9
S	627(72)	686(50)	9.4	349(62)	409(88)	17.2
P	974(134)	1287(430)	32.1	554(198)	720(342)	30.0
Ca	35.4(5.9)	43.9(7.5)	24.0	27.2(6.0)	28.6(8.1)	5.1
Mg	331(43)	413(42)	24.8	238(41)	289(41)	21.4
K	43.2(5.0)	53.9(3.7)	24.8	47.6(11.2)	55.8(13.0)	17.2
Zn	25.0(3.4)	27.0(4.9)	8.0	27.6(4.1)	35.9(15.3)	30.1

ANOVA probability values

	C	N	S	P	Ca	Mg	K	Zn
CO_2	0.678	0.176	0.254	0.514	0.207	0.067	0.276	0.527
Soil	0.105	0.055	0.003	<0.001	0.025	0.015	0.592	0.419
$CO_2 \times$ Soil	0.502	0.674	0.977	0.247	0.327	0.324	0.824	0.611

[a]Standard deviations in parentheses. All units are g of biomass produced per g of nutrient, except for Zn which is kg of biomass per g.

fertility level was a main effect explaining plant elemental concentration; except for C, plants grown in the low fertility soil had significantly ($P < 0.10$) higher concentrations of N, S, P, Ca, and Mg compared to plants grown in the high fertility soil. Carbon to N ratios of above-ground tissue from plants increased with CO_2 enrichment for both soils, but this effect was not statistically significant. The ratio of N:S declined significantly ($P = 0.039$) with CO_2 enrichment.

Biomass produced per unit of nutrient (also referred in the literature as nutrient use efficiencies) increased with CO_2 enrichment but only Mg exhibited a significant ($P = 0.067$) effect (Table 4). The high fertility soil had significantly ($p \leq 0.055$) greater biomass production per unit of N, S, P, Ca, and Mg higher than the low fertility soil.

Concentrations of ortho-P, citrate, malate and enzyme activities of amidase and asparaginase in roots of *L. latifolium* were similar between CO_2 treatments (Table 5). Of these attributes, only concentration of ortho-P was significantly ($P = 0.044$) affected by soil type being higher in root growth in the low fertility soil.

Soil Attributes

As expected, the high fertility soil had greater concentrations or activities of most soil attributes measured than the low fertility soil (Table 6). Carbon dioxide enrichment did affect availability of some nutrients. Acetate extractable Mg^{2+} was influenced by a significant ($P = 0.063$) $CO_2 \times$ soil interaction; CO_2 enrichment increased Mg^{2+} in the high fertility soil, but decreased Mg^{2+} in the low fertility soil. Available N was influenced by a significant ($P = 0.056$) $CO_2 \times$ soil $\times$ plant interaction; CO_2 enrichment induced greater available N in the planted than control pots in the high fertility soil only. The bicarbonate-extractable pool of available P was significantly ($P = 0.050$) greater in soil exposed to CO_2 enrichment regardless of whether the pots were planted or not. Concentration of soil-solution SO_4^{2-} was affected by a significant ($P = 0.042$) $CO_2 \times$ soil interaction; CO_2 enrichment increased SO_4^{2-} availability in planted and control high fertility soil. A significant ($P = 0.045$) $CO_2 \times$ soil interaction affected concentration of soil-solution Mg^{2+}; CO_2 enrichment reduced available Mg^{2+} in both planted and control pots of the low, but not high, fertility soil. Enzyme activities of amidase ($P = 0.252$), glutaminase ($P = 0.966$), and phosphatase ($P = 0.302$) were not significantly affected by CO_2 treatment.

Discussion

Carbon dioxide enrichment influenced many plant and soil responses of the invasive crucifer *L. latifolium* in both low and high fertility soils, but did not influence amidohydrolase activities nor acid phosphatase activ-

Table 5. Concentration of ortho-P, citrate, malate and amidase and asparaginase activities in roots in response to atmospheric CO_2 enrichment by soil fertility level[a]

Attribute	High fertility soil		Low fertility soil	
	Ambient	Elevated	Ambient	Elevated
Ortho-P (μmol g^{-1})	9.07(1.98)	8.99(3.43)	19.74(12.85)	18.89(5.75)
Citrate (μmol g^{-1})	11.99(0.71)	11.91(0.59)	16.35(2.88)	11.84(0.79)
Malate (μmol g^{-1})	8.02(2.58)	8.43(2.09)	12.66(10.96)	8.56(2.67)
Amidase (μmol g^{-1} hr^{-1})	10.75(3.29)	8.02(2.40)	18.19(17.11)	10.07(6.98)
Asparaginase (μmol g^{-1} hr^{-1})	1.09(0.33)	1.05(0.27)	2.42(2.01)	1.63(1.54)

ANOVA probability values

	Ortho-P	Citrate	Malate	Amidase	Asparaginase
CO_2	0.921	0.354	0.531	0.232	0.279
Soil	0.044	0.170	0.166	0.145	0.127
$CO_2 \times$ Soil	0.806	0.156	0.190	0.270	0.295

[a]Standard deviations in parentheses. All values based on fresh root weight.

ity; thus, hypothesis 1 is rejected. We were unable to find literature citations documenting atmospheric CO_2 relationships with soil amidohydrolase activities. The literature does; however, suggest that CO_2 enrichment can significantly increase soil phosphatase activity (Barrett et al., 1998; Kang et al., 2001; Moorhead and Linkins, 1997). Possibilities that might explain the lack of a phosphatase-CO_2 effect in our study include: (1) the experiment may have been of insufficient time to witness an effect; (2) pot studies may under-evaluate such CO_2 effects; and (3) low organic P availability relative to inorganic P availability in the two soils used. The lack of an amidohydrolase-CO_2 effect is more perplexing because the high fertility soil had significantly more available N upon plant harvest with CO_2 enrichment than at ambient CO_2. Given that this increase in available N must occur through organic matter mineralization, one would expect the activities of some amidohydrolases to be higher with CO_2 enrichment to cleave amide groups into the plant available NH_4^+ form. It is possible that our conservative statistical design is overlooking what are actually statistically significant differences in amidohydrolase activities in response to CO_2 enrichment. Alternatively, N mineralizing enzymes not measured in this study may actually control the kinetics of organic matter mineralization. In either case, the fact that available N in the high fertility soil increased in both planted and unplanted controls suggests that CO_2 enrichment influences the soil microbial community regardless of an interaction with plant root exudation.

Hypothesis 2 is rejected because CO_2 enrichment did not increase citrate and malate in roots of *L. latifolium*. One explanation for this is that the soils used in this study, which represent the range of soil fertility levels being invaded, are not fully representative of soils *L. latifolium* is invading; many invaded soils are saline and/or sodic, contain calcium carbonate, and in general have high nutrient availability (Blank and Young, 2002). We discount this possibility because citrate and malate have been shown to be effective in releasing P bound to Al and Fe mineral surfaces characteristic of soils used in this pot study (Penaloza et al., 2002; Shen et al., 2002). In addition, CO_2 enrichment does not necessarily increase root exudation (Niklaus et al., 2001), even exudation of citrate (Barrett and Gifford, 1999). If this is true for *L. latifolium*, there may be no benefit to this plant in producing higher concentrations of citrate and malate in roots with CO_2 enrichment if it is not to be exuded. Fine root turnover has been shown to increase with CO_2 enrichment (Luo et al., 2001), but given the short-term nature of this study, it is impossible to say if fine root turnover over long time periods with CO_2 enrichment might indeed increase plant contributions of acetate and malate to the soil, and thereby increase P availability.

Carbon dioxide enrichment did not significantly increase pools of available soil Ca which suggests rejection of hypothesis 3. *Lepidium latifolium* uptakes considerable Ca and has been shown to increase levels of soil-solution Ca relative to the grass *Elytrigia elongata* it is competing with, presumably to meet nutritional needs (Blank and Young, 2002). In addition, soil pools of Ca have previously been demonstrated to be affected by CO_2 enrichment (Hagedorn et al., 2002). It is possible that hypothesis rejection is comprom-

Table 6. Selected soil attributes following conclusion of experiment in control and planted pots by soil fertility and carbon dioxide level[a]

	High fertility soil				Low fertility soil			
	+ *L. latifolium*		Control		+ *L. latifolium*		Control	
Attribute	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Ext. Ca^{2+} (mmol kg^{-1})	13.9(0.7)	14.8(4.0)	12.8(1.4)	15.9(4.2)	9.1(2.0)	8.3(0.5)	8.4(0.3)	10.0(4.4)
Ext. K^{+} (mmol kg^{-1})	0.75(0.03)	0.83(0.24)	0.75(0.11)	0.91(0.24)	0.30(0.05)	0.27(0.03)	0.30(0.03)	0.38(0.21)
Ext. Mg^{2+} (mmol kg^{-1})	0.69(0.06)	0.75(0.20)	0.61(0.04)	0.70(0.19)	0.57(0.12)	0.44(0.06)	0.64(0.14)	0.55(0.24)
Net N min. (mmol kg^{-1})	1.72(0.80)	1.65(0.68)	1.21(0.83)	1.28(0.32)	0.13(0.08)	0.05(0.08)	0.01(0.06)	0.07(0.08)
KCl N (mmol kg^{-1})	1.20(0.65)	1.74(0.56)	1.89(0.40)	2.09(0.67)	0.07(0.02)	0.07(0.02)	0.08(0.03)	0.10(0.01)
Bicarb-P (mmol kg^{-1})	0.21(0.04)	0.23(0.09)	0.23(0.04)	0.28(0.06)	0.08(0.02)	0.08(0.02)	0.07(0.02)	0.10(0.04)
Amidase (μmol g^{-1} hr^{-1})	14.16(1.44)	16.58(4.30)	15.18(1.74)	17.60(5.37)	0.85(0.36)	0.71(0.10)	0.79(0.14)	0.82(0.41)
Glutaminase (μmol g^{-1} hr^{-1})	15.60(5.96)	16.07(4.36)	17.12(4.57)	20.14(3.34)	0.68(0.86)	0.62(0.55)	1.10(0.76)	0.46(0.43)
Phosphatase (μmol g^{-1} hr^{-1})	2.65(0.42)	2.78(0.94)	2.92(0.40)	3.76(1.59)	0.45(0.20)	0.41(0.10)	0.33(0.26)	0.41(0.19)
ID SO_4^{2-} (mmol L^{-1})	5.3(3.4)	7.0(3.2)	10.1(2.4)	13.4(10.9)	2.6(0.9)	3.7(2.4)	13.2(4.6)	7.9(1.6)
ID Ca^{2+} (mmol L^{-1})	105(14)	115(27)	128(38)	118(23)	64(23)	53(15)	77(33)	65(23)
ID Mg^{2+} (mmol L^{-1})	3.26(0.49)	3.71(0.76)	3.15(0.83)	7.27(4.08)	7.24(3.00)	5.52(2.07)	7.47(4.22)	3.35(1.76)
Total C (mol kg^{-1})	1.23(0.32)	1.05(0.39)	1.29(0.30)	1.32(0.30)	0.56(0.28)	0.48(0.04)	0.52(0.07)	0.50(0.02)

ANOVA probability values

	Ext. Ca^{2+}	Ext. K^{+}	Ext. Mg^{2+}	Net N min.	KCl N	Bicarb-P	Amidase	Glutaminase.	Phosphatase	ID SO_4^{2-}	ID Ca^{2+}	ID Mg^{2+}	Total C
CO_2	0.161	0.173	0.703	0.588	0.074	0.050	0.252	0.966	0.302	0.871	0.769	0.852	0.378
Soil	<0.001	<0.001	0.013	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.065	0.001	0.190	<0.001
CO_2 × Soil	0.328	0.338	0.063	0.831	0.116	0.367	0.145	0.228	0.677	0.042	0.549	0.045	0.843
Plant	0.745	0.302	0.783	0.120	0.119	0.100	0.513	0.281	0.670	<0.001	0.196	0.725	0.246
CO_2 × Plant	0.182	0.348	0.638	0.836	0.084	0.265	0.892	0.935	0.250	0.296	0.548	0.790	0.330
Soil × Plant	0.800	0.976	0.103	0.291	0.213	0.231	0.590	0.482	0.085	0.432	0.997	0.229	0.193
CO_2 × Soil × Plant	0.938	0.906	0.999	0.691	0.056	0.904	0.852	0.588	0.952	0.071	0.593	0.192	0.635

[a]Standard deviations are provided in parentheses. Abbreviations are as follows: Ext. = pH 7.0 ammonium acetate extractable, Bicarb-P = bicarbonate extractable P, ID = immiscibly displaced which is equivalent to the soil-solution pool.

ised by a conservative statistical design. Indeed, with CO_2 enrichment there was an increase in the acetate-extractable pool of Ca^{2+} and greater plant uptake of Ca^{2+}. Moreover, proper testing of this hypothesis may have required a soil more characteristic of where *L. latifolium* is invading presently.

Hypothesis 4 addressed the influence of CO_2 enrichment on plant-soil relationships of *L. latifolium* to investigate the possibility that CO_2 enrichment is allowing *L. latifolium* to become more competitive. Because our experimental design did not incorporate interspecific competition, a direct assessment of the influence of CO_2 enrichment on competitiveness of *L. latifolium* is not possible. In a study of the invasive species *Centaurea solstitialis*, Dukes (2002) determined that for individual plants, growth was greatly enhanced due to CO_2 enrichment, but in competition with representative grass species, the competitive advantage incurred was relatively small. As a generalization, CO_2 growth enhancement measured on individual plants will not proxy for those same plants grown in interspecific competition (Poorter and Navas, 2002). Moreover, CO_2 stimulated growth enhancement may be a poor predictor of competitive success compared to other attributes such as increases in cover (Stewart and Potvin, 1996) and the ability to fix N (Poorter and Navas, 2002). Nonetheless, this study provides evidence that CO_2 enrichment interacts with the plant and soil in particular ways which suggests the possibility of strengthened competitiveness. Carbon dioxide enrichment significantly increased the available pool of soil N (high fertility soil only) and increased the bicarbonate pool of extractable P. Previous field and greenhouse experiments suggest that N and P availability are critical in explaining success of *L. latifolium* (Blank et al., 2002; Blank and Young, 2002). Such increases in availability of these nutrients, especially in nutrient poor environments, may be sufficient to tip the competitive advantage in favor of *L. latifolium*. Carbon dioxide enrichment provides another potential competitive advantage to *L. latifolium* by allowing greater accumulation of plant biomass per unit of nutrient (nutrient use efficiency), which is reported in the literature for many plant species, with some exceptions (Baxter et al., 1994; Davey et al., 1999; Fangmeier et al., 1997; Hagedorn et al., 2002; Johnson et al., 1995). Any competitive benefit *L. latifolium* receives would of course be mitigated by the relative increase of biomass to nutrient uptake of competing plants. Even so, a plant that can produce greater biomass using less nutrients and can increase competitive attributes such as shading ability, seed production, rhizome formation etc. would, as a first approximation, be more competitive. A confounding factor in judging the increased competitive ability of *L. latifolium* to CO_2 enrichment is determining how increasing atmospheric CO_2 has affected, and may continue to affect, the plant-soil relationships of *L. latifolium* along the continuum from pre-industrial to predicted future levels of CO_2. For example, some weedy species have gained greater competitive benefits with increases in CO_2 from pre-industrial to present-day concentrations compared to benefits that are expected to result in future CO_2 enriched environments (Ziska, 2003).

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