Response to elevated CO₂ in the temperate C3 grass *Festuca arundinacea* across a wide range of soils

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Soils vary widely in mineral nutrient availability and physical characteristics, but the influence of this variability on plant responses to elevated CO₂ remains poorly understood. As a first approximation of the effect of global soil variability on plant growth response to CO₂, we evaluated the effect of CO₂ on tall fescue (*Festuca arundinacea*) grown in soils representing 10 of the 12 global soil orders plus a high-fertility control. Plants were grown in small pots in continuously stirred reactor tanks in a greenhouse. Elevated CO₂ (800 ppm) increased plant biomass in the high-fertility control and in two of the more fertile soils. Elevated CO₂ had variable effects on foliar mineral concentration—nitrogen was not altered by elevated CO₂, and phosphorus and potassium were only affected by CO₂ in a small number of soils. While leaf photosynthesis was stimulated by elevated CO₂ in six soils, canopy photosynthesis was not stimulated. Four principle components were identified; the first was associated with foliar minerals and soil clay, and the second with soil acidity and foliar manganese concentration. The third principle component was associated with gas exchange, and the fourth with plant biomass and soil minerals. Soils in which tall fescue did not respond to elevated CO₂ account for 83% of global land area. These results show that variation in soil physical and chemical properties have important implications for plant responses to global change, and highlight the need to consider soil variability in models of vegetation response to global change.

Keywords: soil taxonomy, soil orders, elevated CO₂, *Festuca arundinacea*, tall fescue

INTRODUCTION

Substantial attention has been given to the effects of elevated CO₂ concentration on plant growth and physiology (Körner, 2006), reflecting concern about the performance of both cultivated and wild plants in future climates characterized by elevated CO₂ (IPCC, 2007a). Studies of the responses of crops and ecosystems to elevated CO₂ (Körner, 2006; Ziska and Bunce, 2006, 2007) often report increased growth and use efficiency of nitrogen and water when nutrient availability is optimal or near optimal (Woodward et al., 1991; Bunce, 2004). Though the majority of studies consider elevated CO₂ in isolation, plant responses to elevated CO₂ may be affected by other environmental factors, including soil properties (Diaz et al., 1993; Bassirirad et al., 2001; Spinnler et al., 2002; Lynch and St. Clair, 2004, 2010; Fay et al., 2009, 2012a).

When suboptimal nutrient availability has been considered (generally as deficiency of either N or P), a commonly observed response is that a limited supply of N or P leads to a reduction in photosynthetic rates and foliar N concentrations and increased concentrations of non-structural carbohydrates (NSC) (van Noordwijk et al., 1998; Gifford et al., 2000; Gifford, 2004). Few studies have considered multiple nutrient stresses (deficiencies and/or toxicities) in conjunction with elevated CO₂ (Körner, 2006), and our understanding of the mechanisms behind the interaction of soil characteristics with elevated CO₂ is far from complete (Lynch and St. Clair, 2004). In contrast to research with crops and model plants, forestry and ecological research has considered the effects of elevated CO₂ in natural soils without amendments or fertilizers. Such studies generally indicate multiple and complex limitations, mostly of edaphic origin, that trees face under elevated CO₂ (Bucher-Wallin et al., 2000; Bassirirad et al., 2001; Egli et al., 2001; Poorter and Perez-Soba, 2001; Spinnler et al., 2002).

Natural soils vary widely across terrestrial ecosystems; the USDA soil taxonomy system addresses this variability by classifying soils into 12 orders based on factors related to soil formation (Wilding, 2000; Table S1). These are further divided into suborders and additional sub-categories based on climatic and edaphic modifiers (Soil Survey Staff, 1999). One purpose of such taxonomic systems is to optimize land use over the range of soils so as to maximize productivity and sustainability (Driessen and Konijn, 1992). The variability described by soil taxonomy may also be useful in understanding climate change effects, such as the effect of elevated CO₂ on plant growth, but there has been little effort made to test whether, and to what extent, the effects of elevated CO₂ on plant growth depend on soil taxonomic variation.

A literature search performed on combinations of “Elevated CO₂” and edaphic and soil taxonomy terms produced a relatively small number of hits (Table S2 in Supplemental Materials)—
only 57 unique publications were returned by searching “Elevated CO2 AND soil type” and “Elevated CO2 AND (any USDA soil order)” on Web of Science. Further analysis of topics for the 57 unique publications highlight the scarcity of consideration of the range of possible soil impacts on CO2 responses (Tables S3, S4 in Supplemental Materials). Only 21 of these publications report results from an experiment with two or more soils and elevated CO2, and the maximum number of soils considered was three. Furthermore, these 21 publications report on only 6 unique experiments, and 16 of the 21 reports and 3 of the 6 unique experiments relate to woody plants. Although several authors have noted the importance of soils in determining plant responses to elevated CO2 (Bassirirad et al., 2001; Pootter and Perez-Soba, 2001; Spinnler et al., 2003; Fay et al., 2009, 2012a), it appears that no attempt has been made to test the extent to which plant responses to elevated CO2 vary across the natural variability of soils.

As noted above, work on woody plants may be more advanced than work in non-woody plants in this area. Watanabe et al. (2013) reported no CO2 × soil interaction on photosynthetic traits of hybrid Larix grown for two seasons on a fertile forest soil and an infertile volcanic ash soil. In contrast, Spinnler et al. (2002) found that while in Picea there was no CO2 × soil interaction on biomass, in Fagus elevated CO2 only stimulated growth in a more fertile calcareous soil, and actually suppressed growth on an acidic soil. In the same system it was found that responses to elevated CO2 may differ between root and shoot (Sonnleitner et al., 2001). Such differences may have ecosystem consequences; another report on the same system showed that the acidic soil increased its carbon content to a much greater degree than the calcareous soil, even though it supported much less biomass (Hagedorn et al., 2003). Interestingly, while biomass responses of Picea and Fagus in this system were strongest in the early years of this 4 year study, plant water relations still responded to elevated CO2 in a soil-dependent manner (Bucher-Wallin et al., 2000). In contrast, another study showed no effect of elevated CO2 on stomatal conductance or leaf hydraulic conductivity in Betula or Quercus grown on two contrasting soil, though responses to CO2 differed between sun and shade leaves (Eguchi et al., 2008).

The best examples for non-woody plants are a series of reports on experiments carried out with monoliths of three soils that were exposed to a CO2 gradient from sub- to super-ambient. In this system early growth of Panicum virgatum was enhanced by elevated CO2 but not regrowth after clipping. An interaction of Soil × CO2 was seen for soil moisture but not for annual net primary productivity (Fay et al., 2012b). Another study using this system with constructed prairie plant communities found that aboveground biomass response to CO2 was greatest on soils with greater plant available water (a Mollisol and an Alfisol) and was reduced on a heavy clay soil (a Vertisol) with lower plant available water (Fay et al., 2012a). Another report on this system showed that in the Mollisol forbs responded more strongly to elevated CO2 than grasses, though grasses were stimulated by increasing CO2 in all three soils (Polley et al., 2012).

Grasses (Poaceae) include cereal crops that provide over half of the calories and protein consumed by humans (Cordain, 1999) and are the principle vegetation of the 30% of global land area occupied by natural grasslands (Bartholome and Belward, 2005; Lambin and Geist, 2006). Grasses represent a large, variable group of plants that are successful in many environments and that have evolved several mechanisms to adapt to extreme soil conditions (Marschner, 1998).

In order to begin to characterize the effects of the wide range of soils on plant responses to elevated CO2 we grew tall fescue (Festuca arundinacea Schreb.), a temperate C3 grass, in elevated and ambient CO2 on 13 different soils, representing ten of the twelve soil orders, and a high fertility control, and assessed plant growth, mineral acquisition, gas exchange, and non-structural carbohydrate accumulation.

**MATERIALS AND METHODS**

**EXPERIMENTAL SETUP**

Tall fescue was grown in eight Continuous Stirred Tank Reactors (CSTRs; mylar covered cylindrical steel frames approximately 2 m in diameter and 2 m tall with a continually rotating stirring paddle near the top to ensure even mixing of the atmosphere; Heck et al., 1975) in a greenhouse at the Pennsylvania State University (40°08’N, 77°83’W). The CSTRs were covered in transparent mylar and fitted with a positive pressure ventilation system that provided an airflow of 1 L per minute to each CSTR. Each CSTR was equipped with an external overhead 1000 watt HID Lamps for supplemental light; maximum light intensity at plant level averaged 350 µmol PAR s^-1 m^-2 (This relatively low lighting intensity reflects both the attenuation of solar radiation through both greenhouse roof and the mylar covering of the CSTRs and the difficulty of controlling heat load from the HID lighting supplied to each CSTR).

The eight CSTRs were grouped into four pairs, with one of each pair receiving near ambient (400 ppm CO2, which was near the ambient level in the greenhouse) and the other receiving elevated (800 ppm CO2, corresponding to the IPCC’s “worst case” A1FI scenario for mid; IPCC, 2007b). Elevated CO2 was maintained by bleeding 99.8% dry CO2 from a pressurized tank via a needle valve into a manifold from which four valves controlled the flow of CO2 to each of the elevated CO2 CSTRs. These valves were adjusted daily to maintain the target CO2 concentration. CO2 concentration (measured with a Li-Cor 6262 infrared gas analyzer connected to a multiplexing pump), temperature, photosynthetically active radiation (PAR), and relative humidity for each CSTR were recorded every 16 min. CO2 concentrations were relatively stable, with mean values (±1 standard deviation of 790 ± 14 ppm for Elevated CO2 and 399 ± 11 ppm for near ambient CO2).

**PLANTING**

Soil samples representing 10 taxonomic orders (Table 1) were obtained from Puerto Rico, Ecuador and the U.S. (Alaska) during 2005 and 2006. In each location we collected from areas with no known history of fertilizer use. Soils were air dried and transported to University Park, PA, where they were kept in refrigerated storage (6°C) until the experiment began in January 2007, when the soils were sieved (2 mm) to exclude gravel and organic debris, and eight pots (400 ml volume) were filled with each soil type. Pots were also prepared with a high-fertility
Table 1 | Properties of soils used in the study.

| Soil ID | Order (suborder) | Origin | pH  | Total N (%) | P (ppm) | K (ppm) | Mg (ppm) | Ca (ppm) | Zn (ppm) | Cu (ppm) | S (ppm) | Clay% | Silt% | Sand% |
|---------|------------------|--------|-----|-------------|--------|--------|----------|---------|---------|---------|--------|------|------|------|
| ALF     | Alfisol (Udalf)  | PR     | 6.4 | 0.33        | 7      | 210    | 147      | 2065    | 3.6     | 4.4     | 46     | 15.3 | 22.3 | 62.3 |
| AND     | Andisol (Aquand)| EC     | 5.4 | 0.47        | 12     | 148    | 73       | 439     | 3.7     | 5.9     | 29.3   | 1.06  | 28.4 | 70.5 |
| ARD     | Andisol –        | EC     | 7.9 | 0.09        | 71     | 182    | 633      | 2582    | 4.2     | 9.4     | 36.2   | 8.06  | 41.5 | 50.4 |
| INC1    | Inceptisol (Tropent) | PR | 5.6 | tr          | 7      | 86     | 168      | 768     | 3.5     | 3.1     | 19.6   | 0.45  | 5.46 | 94.1 |
| INC2    | Inceptisol (Udept) | PR  | 4.7 | 0.05        | 1      | 84     | 17       | 120     | 0.3     | 3.8     | 216    | NA   | NA   | NA   |
| GEL     | Gelisol –        | AK     | 7.7 | 0.19        | 9      | 50     | 164      | 5490    | NA      | NA      | NA     | 12.1  | 59.7 | 28.2 |
| MOL     | Mollisol (Ustol) | PR     | 7.5 | 0.16        | 200    | 689    | 497      | 5001    | 4.5     | 10.2    | 22.4   | 30.1  | 31.4 | 38.5 |
| OX1     | Oxisol (Ustox)   | PR     | 7.7 | 0.21        | 11     | 280    | 124      | 2987    | 1.7     | 5.2     | 177    | 26.0  | 29.2 | 44.8 |
| OX2     | Oxisol (Uadox)   | PR     | 5.2 | 0.15        | 1      | 44     | 57       | 189     | 0.7     | 1.4     | 285    | 4.1   | 11.6 | 84.3 |
| OX3     | Oxisol –         | EC     | 5.8 | 0.26        | 9      | 28     | 52       | 522     | 1.4     | 2       | 18.9   | 13.0  | 29.1 | 579  |
| SPO     | Spodosol (Orthod) | PR    | 7.3 | 0.19        | 20     | 33     | 125      | 3566    | 19.4    | 13.6    | 29.5   | 1.56  | 11.1 | 87.3 |
| ULT     | Ultisol (Humult) | PR     | 5.4 | 0.29        | 14     | 545    | 225      | 1248    | 2.6     | 6.4     | 44.3   | 20.8  | 33.2 | 46.1 |
| VRT     | Vertisol (Ustert)| PR     | 6.8 | 0.15        | 10     | 150    | 1737     | 5016    | 1.4     | 8       | 23.3   | 22.9  | 29.7 | 47.4 |

PR, Puerto Rico; EC, Ecuador; AK, Alaska (United States); NA, Not available; tr, trace values.

count control treatment (“CTR”), consisting of a standard horticultural medium based on peat and vermiculite (Sunshine Mix #3, Sun Gro Horticulture, Bellevue, WA) amended with a complete slow-release fertilizer (Osmocote 14-14-14 in a rate of approximately 3 g per pot; Scotts Miracle-Gro, Marysville, OH).

Approximately 20 seeds of tall fescue (cultivar Kentucky 31; SeedLand, Inc. Wellsborn, FL) were broadcast on the surface of each pot on Jan 23, 2007. The pots were covered in clear plastic until germination, after which pots were thinned to 15 plants pot\(^{-1}\). 14 pots (the 13 soils plus the fertile control) were randomized within each of the eight CSTRs, for a total of 112 pots. Pots were irrigated manually with distilled water every day.

**DATA COLLECTION**

The presence of the endophyte *Neotyphodium sp.* was assessed from a sample of 100 seeds and two growing tillers per pot (from two replicates), using a commercial immunoblot detection kit (Agrinostics Ltd. Co., Watkinsville, GA).

Leaf photosynthesis (\(A_{\text{max}}\), area basis) of a young, fully expanded leaf in one plant per pot was determined on weeks 8 and 10 at mid-day with a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE) with leaf temperature set to 20°C. Measurements were made at 600, 800, and 1000 µmol m\(^{-2}\) s\(^{-1}\) PAR, after measurements at 200–1000 µmol m\(^{-2}\) s\(^{-1}\) PAR on a subset of plants showed that maximum photosynthetic rate occurred in the 600–1000 µmol m\(^{-2}\) s\(^{-1}\) range. Also at week 10 the net pot CO\(_2\) exchange (including soil) was measured with a Li-6200 Infrared Gas Analyzer system (Li-Cor, Lincoln, NE) using a 12 liter chamber in which the whole plant and pot were enclosed for 2 min. During these measurements temperature ranged from 25 to 28°C, and light (PAR) ranged from 320 to 380 µmol m\(^{-2}\) s\(^{-1}\), reflecting the ambient growing conditions.

Above-ground tissue was harvested following CO\(_2\) exchange measurement. Immediately following shoot excision, the same method was used to measure the amount of respiration from the roots and soil. Canopy CER was estimated as the difference between the net pot CO\(_2\) exchange and root + soil CO\(_2\) exchange.

Excised shoots were divided into three samples. The first sample (~5 g fresh weight) was frozen at –80°C. These were later processed to quantify ethanol soluble sugars and starch from approximately 100 mg of ground tissue with the enzyme-coupled colorimetric method described by Hendrix (1993).

All remaining above-ground tissue was oven dried at 60°C for 48 h to determine total dry weight. A sample of about 0.2 g of dried and ground leaf tissue was digested in a microwave (Miller, 1998). The diluted (250:1) extract was analyzed in a Varian Induced Couple Mass Spectrophotometer (Varian Inc., Palo Alto CA) to determine the content of: phosphorus, potassium, calcium, magnesium, manganese, iron, copper, boron, aluminum, zinc and sodium. A second sample of dried and ground tissue
was analyzed using a Perkin-Elmer EA2400 elemental analyzer with combustion and reduction columns to determine carbon and nitrogen content.

STATISTICAL ANALYSIS
The data were analyzed with a split-plot design. The four pairs of CSTRs were treated as blocks, with CO2 as the main plot factor and soil the subplot factor. Data were analyzed in R (R Core Team, 2014) using lme (Pinheiro et al., 2014), to fit linear mixed effects models with block and CSTR as random effects (The model was “response ~ CO2 + Soil + Soil:CO2, random = ~1|Block/CSTR”), and residuals were checked to ensure that regression assumptions were not violated. For $A_{\text{max}}$, the mixed effect model included all saturating levels of PAR with PAR as a random effect (The model was “$A_{\text{max}}$ ~ CO2 + Soil + Soil:CO2, random = ~1|Block/CSTR/PAR”). Pair-wise Tukey comparisons for the effect of CO2 in each soil were obtained using multcomp (Hothorn et al., 2008).

Given the large number of variables measured (31) Principal component analysis (PCA) was used to characterize the principal sources of variability in the data. PCA was carried out for all variables measured using prcomp (R Core Team, 2014).

RESULTS
About 40% of the seeds and 95% of all tillers tested positive for the Neotyphodium endophyte, and Neotyphodium colonization did not differ among soils or CO2 treatments.

Of the soils used in this experiment, only INC2 did not support any germination of tall fescue. Plants grown in GEL, HIS and OXI2 exhibited severe reductions (>97%) in biomass compared to CTR, and did not produce sufficient tissue for all analyses to be completed. They were therefore eliminated from most of the subsequent analyses. The remaining soils produced 50–80% less shoot biomass than CTR, indicating significant differences among soils (Table 2). These differences were especially notable under elevated CO2, where biomass of CTR increased by about 60% (Figure 1A). Elevated CO2 significantly increased plant biomass in the CTR, ULT and ALF (by approximately 20–40%), but did not increase biomass in the other soils (Figure 1A).

In the soils used in this experiment elevated CO2 did not significantly change leaf nitrogen concentration ($p = 0.323$; Figure 1B, Table 2), though there were differences in leaf N associated with the soils ($p < 0.001$), as would be expected. Furthermore, the C:N ratio was not altered by CO2 or the CO2 × soil interaction ($p > 0.300$, data not shown), though it did differ among the soils ($p < 0.001$, Table 2).

Leaf phosphorus concentration declined approximately 30% with elevated CO2 in CTR, while in the remaining soils phosphorus concentration changed only marginally ($p = 0.062$; Figure 2A). Leaf potassium concentration was reduced by 40% under elevated CO2 in ALF soil, and increased by 100% in INC1, but was not affected in the remaining soils ($p > 0.650$; Figure 2B). Leaf K concentration was dramatically lower in SPO than in CTR, but the remaining soils produced leaf K values within about ±20% of CTR (Figure 2B).

Table 2 | Summary of analysis of variance results of biomass, mineral content, and gas exchange parameters for Festuca arundinacea var.

| Source          | df | F-value | df | F-value | df | F-value | df | F-value | df | F-value |
|-----------------|----|---------|----|---------|----|---------|----|---------|----|---------|
| Biomass         |    |         |    |         |    |         |    |         |    |         |
| CO2             | 1/3| 6.98*   | 1/3| 7.71+   | 1/3| 1.01    | 1/3| 7.29+   |    |
| Soil            | 13/77| 117*** | 10/208| 15.5*** | 10/60| 10.8*** | 19.1*** |
| CO2 × Soil      | 13/77| 5.50*** | 10/208| 6.57*** | 10/60| 0.903   | 2.24*   |
| N               |    | 2.12    |    | 1.13    |    | 15.8*   |    | 3.01    |    |
| C:N             |    | 25.5*** |    | 7.50*** |    | 9/54    | 2.05+ | 7.29**  |
| P               |    | 1.32    |    | 0.696   |    | 9/54    | 1.25  | 0.711   |
| K               |    |         |    |         |    |         |    |         |    |
| Ca              |    |         |    |         |    |         |    |         |    |
| Mg              |    |         |    |         |    |         |    |         |    |
| Mn              |    |         |    |         |    |         |    |         |    |
| Pot Resp.       |    |         |    |         |    |         |    |         |    |
| Shoot CER       |    |         |    |         |    |         |    |         |    |
| CO2             | 1/3| 8.43+   | 4.51| 18.9    | 15.8*| 11.6*   |
| Soil            | 10/56| 55.1*** | 20.7*** | 72.6*** | 26.7*** | 126*** |
| CO2 × Soil      | 10/56| 3.26** | 3.70*** | 3.08** | 1.78+ | 1.75+   |
| Al              |    |         |    |         |    |         |    |         |    |
| Fe              |    |         |    |         |    |         |    |         |    |
| B               |    |         |    |         |    |         |    |         |    |
| Na              |    |         |    |         |    |         |    |         |    |
| Zn              |    |         |    |         |    |         |    |         |    |

Kentucky 31 grown in ambient (400 ppm) and elevated (800 ppm) CO2 in 13 different soils plus a high-fertility control.*

* Not all soils produced sufficient biomass for all analyses, leading to differences in degrees of freedom between analyses. Degrees of freedom (df) printed to the left of each variable and listed as numerator df/denominator df. Pot Resp. = Root + Soil CO2 exchange rate. Significance denoted as: *+$p = 0.1–0.05$; **+$p = 0.05–0.01$; ***+$p = 0.01–0.001$; ****+$p < 0.001$. 
The ANOVA analysis for leaf mineral concentrations found highly significant differences ($p < 0.001$) for the soil effect for all leaf nutrient concentrations measured. However, the results for CO$_2$ and the soil $\times$ CO$_2$ interaction were less consistent. For example the soil $\times$ CO$_2$ interaction was significant for P, K, Ca, and Zn, and only marginally significant for Mg, Mn, and Fe. (Table 2). Elevated CO$_2$ increased content of calcium in INC1 and SPO ($p < 0.001$), of magnesium in CTR ($p = 0.038$) and
Elevated CO₂ increased leaf photosynthesis ($A_{\text{max}}$, μmol CO₂ s⁻¹ m⁻² leaf area) in most of the soils, but not in CTR, ALF, MOL, and OXI3, reflecting a significant soil × CO₂ interaction ($p < 0.001$; Table 2, Figure 3A). Among the soils $A_{\text{max}}$ in ambient CO₂ was highest in CTR and AND in ambient and elevated CO₂ respectively and lowest in INC1 (both CO₂ levels; Figure 3A, Table 2).

Aboveground carbon exchange rate (Shoot CER; nmol CO₂ s⁻¹ mg⁻¹ DW canopy) did not differ significantly between elevated and ambient CO₂ overall ($p = 0.389$), but varied between soils ($p < 0.001$), and there was no interaction between CO₂ and soil (Table 2, Figure 3B). Root + soil CO₂ exchange rate (nmol CO₂ s⁻¹) increased in CTR, ALF, ARD, and MOL, but not in the others (Figure S1), leading to a significant Soil × CO₂ interaction ($p = 0.027$; Table 2).

Combining CO₂, biomass production, mineral content and photosynthesis variables we created a matrix of 29 variables and 76 observations after records with missing data were excluded (mostly soils in which insufficient biomass was produced for all analyses to be completed; HIS, GEL, INC2, and OXI2 were excluded. Since % Sand, % Silt, and % Clay sum to 100%, we excluded % Sand from this analysis. Similarly, since CEC is reflective of soil Ca and Mg, we excluded CEC.

Principal components analysis yielded four principal components (PC) which explained about 65% of the variability in the data (Figure 4). PC1 (29% of variability) was most strongly influenced by foliar mineral concentrations (Zn, Cu, Mg, Ca, and Na) and soil clay content (minerals declining with increasing clay). PC2 (19% of variability) was most influenced by soil copper, soil calcium, soil pH foliar P and foliar Mn concentrations (all others decrease when foliar Mn increases). PC3 (9% of variability) was most strongly influenced by gas exchange (leaf photosynthesis and stomatal conductance) and foliar Al, P, Fe and C (photosynthesis increasing with decrease in foliar minerals). PC4 (8% of variability) was most strongly influenced by above-ground biomass, sucrose, soil S, soil K, and Mg (with Mg decreasing when others increase; Table S5).

High and low molecular weight NSC (starch and sucrose respectively) responded differently in this experiment (Table 2). Starch concentrations increased under elevated CO₂ ($p = 0.029$), but sucrose concentrations were not affected by CO₂ ($p = 0.181$). Levels of NSC were influenced by the different soils ($p = 0.051$ and 0.009 for starch and sucrose respectively; Table 2). However, the effect of elevated CO₂ on NSC did not depend on soil type ($p = 0.288$ and 0.697 for starch and sucrose respectively; Table 2, Figure 5).

Maximum photosynthetic rate ($A_{\text{max}}$) did not show a strong correlation with foliar N (Figure 6A). Furthermore, the relationship between $A_{\text{max}}$ and foliar N differed between soils (0.0008) and marginally with CO₂ (0.079). A graphical analysis (based on non-overlap of SE ellipses, Figure 6A) suggests that for OXI1, ULT, SPO, CTR, and AND $A_{\text{max}}$ may not be strongly related to foliar N. The tukey test of pairwise differences confirms this for OXI1 and ULT ($p = 0.006$ and 0.062).

Analysis of N:P ratios (Figure 6B, Table 2) shows a strong effect of soil ($p < 0.001$) but no effect of CO₂ ($p = 0.410$), or their interaction ($p = 0.131$). Three rough groupings of soils are differentiated here. CTR and MOL, with a low N:P ratio, INC1, AND, and OXI3, with a high N:P ratio, and ARD, ALF, OXI1, SPO, ULT, and VRT with intermediate values.

**DISCUSSION**

In this experiment we evaluated the effect of elevated CO₂ on the growth and physiology of *Festuca* encountering different chemical and physical soil characteristics presented by soils from 9 of the 12 soil orders, spanning the global range of soil

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**FIGURE 3** | Leaf photosynthesis of individual leaves (A) and net carbon exchange rate (CER) for the shoot (B) of *Festuca arundinacea* grown in 10 soils, plus a high-fertility control under elevated (800 ppm; red) and ambient (400 ppm; blue) atmospheric CO₂. Mean of four replicates ± one standard error shown. The soils are ordered by decreasing biomass in ambient CO₂, as in Figure 1A. *p = 0.05–0.01; **p = 0.01–0.001; ***p < 0.001.*
variability. The effect of elevated CO2 on growth, photosynthesis, and leaf chemistry depended on the soil in which the plants were grown. Since we provided adequate irrigation, we assume that the responses we observed mostly reflect the ability of Festuca to acquire nutrients from the different soils under contrasting CO2 regimes.

Of the 13 soils used in this experiment four soils either failed to permit germination or to produce sufficient tissue for all of our assays. These are extreme soils for which Festuca, despite its wide range of adaptability (Malinowski and Belesky, 2000; Rahman and Saiga, 2007), is apparently not well adapted. These soils include the two soils with the lowest phosphorus values (INC2 and OX12) and the lowest pH of all the soils (INC2).

Although Neotyphodium is restricted to the aerial parts of the plant, its presence has been related to increased tolerance of fescue to several edaphic stresses, including P and Ca, (Malinowski et al., 2000) though the effect of endophyte infection may depend on plant genotype and soil (Rahman and Saiga, 2007). We found no effect of soil or CO2 on colonization of Neotyphodium in fescue; we therefore do not expect Neotyphodium colonization to favor specific soil treatments (Malinowski and Belesky, 2000).

We found large differences between soils in the effects of elevated CO2 on Festuca. First, there was a large increase in biomass.
under elevated CO₂ on soils where biomass was greater under ambient CO₂, in contrast to the lack of stimulation seen in soils that supported less biomass production in ambient CO₂. To some extent, this may simply indicate that soils where Festuca grows well support a greater increase in biomass in elevated CO₂; i.e., there may be a correlation between biomass increase in elevated CO₂ and biomass in ambient CO₂. This relationship is significant, but only explains 44% of the variability in CO₂ response ($p < 0.001, R^2 = 0.44$). This suggests that other factors may also be important.

Our results generally agree with previous reports of the lack of response to increased CO₂ under nutrient-limited conditions (Poorter and Perez-Soba, 2001; Ziska and Bunce, 2006). Differences in leaf elemental concentration highlight a second important response; some soils have inherent low levels of N, P and K, and plants do not accumulate these elements to sufficient levels, and therefore may experience limitation by these elements. For example plants grown in Spodosols had very low foliar K (Figure 2B), suggesting K limitation as a possible cause for the low biomass production (Figure 1A) in this soil. In some cases elevated CO₂ led to the accumulation of non-limiting elements. For example under elevated CO₂ plants in AND and INC1 had higher levels of foliar N than CTR or any other more fertile soil; plant growth in these soils was apparently not limited by nitrogen, but the very low biomass of these plant points at some limitation (Figure 1).

The carbon exchange rate for whole shoot and leaf photosynthesis (µmol CO₂ m⁻² leaf s⁻¹) also showed contrasting results (Figure 3). While leaf photosynthesis ($A_{max}$) showed the expected increase in response to elevated CO₂ in six of the soils, shoot CER was not altered by elevated CO₂. The difference between leaf and canopy photosynthetic responses to elevated CO₂ was not a simple product of changes in biomass as shoot CER was normalized by biomass. There was a significant negative relationship (log-linear) between shoot CER and shoot biomass (Figure 6A, $p < 0.001, R^2 = 0.30$). However, shoot CER was positively related (log-linear) with leaf N (Figure 6B, $p < 0.001, R^2 = 0.17$). There are reports of growth dilution of leaf N by elevated CO₂ (Luo et al., 1994; Idso and Idso, 2001; Taub and Wang, 2008; Wieser et al., 2008), but since we saw no difference in leaf N (Figure 1B) or C:N ratio (not shown) with CO₂ there is no evidence of growth dilution. While some of the difference between leaf and canopy level responses may be explained by the lower light levels for the measurement of CER relative to that used for the leaf photosynthesis (300 vs. 10000 µmol PAR s⁻¹ m⁻²), the relationship of leaf N concentration and canopy CER suggests that there are fundamental differences in photosynthetic N use between plants grown in different soils.

In a review of research on N:P ratios, Güsewell (2004) reported that N:P ratios near 13 were typical for plants grown in their native conditions. In our study, only ALF, ULT and VRT under elevated CO₂ showed values near this (Figure 7B). A range of 9–19 for N:P ratios was also reported for a range of plants in a range of vegetative communities (Güsewell, 2004), with a range of 10–14 for graminoids. As shown in Figure 7B, few of our plants fell within this range (ALF, ARD, and ULT in elevated CO₂, and VRT in both atmospheres). Without more detail on the soil the reported N:P ratios represent it is difficult to interpret these results—it is possible that the range of N:P ratios reported does not represent the range of soil variability we are testing here. Alternatively, the wide range of N:P ratios we report here may indicate that fescue is not well adapted to some of these soils. However, correlation between the divergence of observed N:P ratio from the “optimum” value of 13 and biomass was very low ($r = -0.25$) suggests that divergence of N:P ratio from some optimum is not strongly related to biomass.

The progressive nitrogen limitation hypothesis (Luo et al., 2004) suggests that increased plant biomass (and hence soil organic matter) stimulated by elevated CO₂ can immobilize sufficient N to lead to increasing N limitation with elevated CO₂. We saw little support for this in this study. Foliar N had limited influence on $A_{max}$ (Figure 7A). Furthermore, biomass was stimulated in only 3 soils (Figure 1A) and root respiration was only...
modestly increased in four soils (of which two showed above-ground biomass increase also). So in 6 of the 11 soils for which we could fully analyze results there was no stimulation of biomass or below-ground respiration. We note however that the progressive nitrogen limitation hypothesis is likely to operate more strongly on ecosystem spatial scales and over multiple seasons, as a key mechanism for progressive limitation is that a greater proportion of N ends up in plant tissue and in soil organic matter. In this study, the elevated CO2 treatment was confined to 10 weeks, which is very unlikely to be a sufficient time-span for progressive limitation to occur to a notable extent.

A substantial number of studies have considered the effect of elevated CO2 on root:shoot allocation. We did not harvest roots in this study because the relatively small sizes of plants in this study mean that roots will mostly be rather fine. This fact, combined with the wide range of soil textures (Table 1) would not have yielded reliable data, as the recovery rate for roots would have varied rather strongly with soil texture.

An increase in non-structural carbohydrates (NSC) as been reported in plants exposed to elevated CO2 (Poorter and Perez-Soba, 2001; Ziska and Bunce, 2006, 2007). The pattern of accumulation of NSC we observed suggests that carbohydrate physiology may be strongly influenced by differences in soils, though the variability in NSC was rather high (Figure 5). Accumulation of NSC in elevated CO2 in the high-fertility control was not significant, while in ALF and AND there was accumulation of both high- and low-weight NSC. In ULT, ARD, and MOL only high weight NSC accumulated. In contrast, for SPO high weight NSC were reduced in elevated CO2. The lack of accumulation in SPO, OXI3 and VRT suggests active use of carbon in the plant, possibly in high metabolic demand processes such as mineral acquisition. Alternatively, carbon losses could take place through respiration or rhizo-deposition (Nguyen, 2003). These contrasting responses highlight the ways in which carbohydrate assimilation and metabolism may be influenced by soil conditions.

The four principal components we found in this study improve our understanding of the inter-relationships among mineral content in soils, foliar concentration of minerals, photosynthesis and biomass (Figure 4). In the soils we sampled, foliar levels of Zn, Cu, Mg, Ca, and Na tended to be higher in soils with lower clay content (PC1). In general cations such as Mg and Ca are more available in soils with greater cation exchange capacity, which tend to be soils with higher clay and organic matter content. However, here we see a tendency for lower soil clay to be associated with higher values of these minerals in leaves, suggesting that soil availability of these minerals is not the strongest determinant of their foliar concentration. Spodosols were differentiated on PC1, likely due to the high concentration of calcium and sodium observed in leaves and the low clay content. The high Na content in SPO may have reduced K availability and altered carbohydrate physiology as reflected in the distinct NSC patterns in SPO discussed above. In most soils we observed a decline in the values of PC1 with elevated CO2, suggesting a trend of dilution of minerals as was observed clearly with CTR, ALF and ULT. The risk of mineral dilution and the consequent loss of food and forage quality has been mentioned by others (Idso and Idso, 2001; Wieser et al., 2008); our findings suggest that this may affect plants on some soils differently than on others.

PC2 was most influenced by soil minerals (Cu, Ca, Zn), soil pH, foliar P, and foliar Mn (with the opposite sign). This is not surprising, as soil pH governs soil mineral availability, and it is well known that in acidic soils low foliar concentrations of P and high concentration of Mn can inhibit growth (Marschner, 1998). On PC3 photosynthesis and stomatal conductance are opposite in sign to foliar Al, P, Fe, and C. This grouping may indicate limitation of growth and photosynthesis by something other than P; in such conditions foliar P might be less correlated to photosynthetic responses. On PC4, the loading of biomass, low molecular weight NSC (sucrose), and foliar C indicate that growth is favored under conditions that favor sucrose, rather than starch, accumulation in leaves. Starch accumulation in leaves is one symptom of severe P deficiency (Marschner, 1998).

The fact that the first four PCs only captured 65% of the variation in the data indicates that the relationships between photosynthesis, leaf mineral content, and soil physical and chemical properties is complex and highly dimensional—a small number of variables will not adequately describe the range of differences seen. The strongest loadings for CO2 were on PC6 (4.7% of the variation) and PC12 (2% of the variation), and CO2 was also loaded on PC5 (5.4% of variation). This suggests that variability associated with CO2 was relatively low in this data set compared with that associated with plant responses to diverse soils. This suggests that more work is needed with highly diverse soils to better map the potential responses of plants to global change variables.

Soil texture and its influence on plant available water has been suggested as a mechanism that mediates differing responses to elevated CO2 (Fay et al., 2012a). The interaction of soil texture and elevated CO2 via water availability is an important mechanism that requires further investigation. Our methodology in this study did not test these responses as all plants were well watered. In natural systems where water availability is limiting, responses to elevated CO2 could be larger than what we observed. However, as noted by Lynch and St. Clair (2004), toxicity of metals such as Mn can be strongly controlled by soil moisture, so it is also possible that increasing soil water could have negative effects on plant growth. Given the importance of this interaction, a more complete exploration of this interaction is clearly needed. In order to avoid artifacts introduced by sieving or mixing soils, such a test would best be achieved using soil monoliths and a method of providing experimental units with the same total water over the growing season.

The differences among soils in the response to elevated CO2 suggest some caution in predicting plant responses to elevated CO2 based on the world-wide network of free-air carbon enrichment (FACE) sites without considering how the FACE sites reflect the global diversity of soils. Such caution has been suggested by others who have noted that the distribution of soils limited by acidity (von Uexkull and Mutert, 1995) and phosphorus deficiency (Sanchez, 1976, 1981; Fairhurst et al., 1999; Jaramillo, 2011) and how these contrast with the geographic concentration of the free-air concentration enrichment (FACE) studies in countries in zones free of these edaphic limitations (Schimel, 2006).
These differences also raise the possibility that models derived from FACE studies on high fertility sites could be overestimating any positive “silver-lining” effect of climate change on food production (Reilly and Schimmmelpfennig, 1999; Long et al., 2006; Leakey et al., 2012).

In this study elevated CO2 increased biomass of Festuca in only ULT, ALF, and in the high-fertility control. ULT and ALF are only present in relatively small areas of the world (Table 3), accounting for 17–25% of land area, depending on continent. Others have noted that CO2 enrichment studies have predominantly reflected temperate biomes, which may respond differently than do tropical or arctic biomes (Leakey et al., 2012). Since Festuca did not germinate on the Gelisols we cannot speculate on the possible response of plants growing in regions from the tundra and other areas with permafrost soils. These frigid zones are the ones that could experience faster and greater impact of the expected temperature increase due to global change (IPCC, 2007a,b). While we could experience faster and greater impact of the expected temperature increase due to global change (IPCC, 2007a,b).

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### Table 3 | Percent area of each of the soil orders in six continents.

| Order   | S. America | Africa | Asia | Oceania | N. America | Europe |
|---------|------------|--------|------|---------|------------|--------|
| Alfisols| 10.2       | 12.3   | 4.90 | 14.8    | 10.4       | 25.4   |
| Andisols| 1.39       | 0.16   | 0.57 | 0.88    | 4.53       | 0.46   |
| Aridisols| 8.22      | 14.0   | 11.8 | 35.1    | 789        | 0.85   |
| Entisols| 15.0       | 41.8   | 11.3 | 26.3    | 6.89       | 6.27   |
| Gelisols| 0.49       | 0.00   | 27.4 | 0.00    | 13.3       | 5.84   |
| Histisols| 0.25      | 0.06   | 1.58 | 0.02    | 2.39       | 2.68   |
| Inceptisols| 11.8      | 6.36   | 26.1 | 4.19    | 25.5       | 19.0   |
| Molisols| 6.43       | 0.35   | 7.75 | 1.65    | 11.0       | 14.5   |
| Oxisols| 30.9       | 13.9   | 0.21 | 1.15    | 0.56       | 0.00   |
| Spodosols| 0.16      | 0.00   | 0.71 | 0.93    | 11.0       | 24.2   |
| Ultisols| 14.2       | 7.35   | 6.48 | 3.56    | 4.30       | 0.04   |
| Vertisols| 0.93      | 3.64   | 1.30 | 11.5    | 2.25       | 0.77   |

Soils in which biomass of Festuca was stimulated by elevated CO2 are indicated in bold.
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