Will rising atmospheric CO_2 concentration inhibit nitrate assimilation in shoots but enhance it in roots of C_3 plants?

Mitchell Andrews[a*], Leo M Condron[a], Peter D Kemp[b], Jennifer F Topping[c], Keith Lindsey[c], Simon Hodge[a] and John A Raven[d,e,f]

[a]Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, New Zealand

[b]Institute of Agriculture and Environment, Massey University, Palmerston North, New Zealand

[c]The Integrative Cell Biology Laboratory, Department of Biosciences, Durham University, Durham DH1 3LE, UK

[d]Division of Plant Science, University of Dundee at the James Hutton Institute, invergowrie, Dundee DD2 5DA, UK

[e]School of Plant Biology, University of Western Australia MO84, 35 Stirling Highway, Crawley, WA 6009, Australia

[f]Climate Change Cluster, University of Technology, Sydney, Ultimo, NSW 2007, Australia

Correspondence

*Corresponding author, e-mail: mitchell.andrews@lincoln.ac.nz

Bloom et al. (2019) proposed that rising atmospheric CO_2 concentrations "inhibit malate production in chloroplasts and thus impede assimilation of nitrate into protein of C_3 plants, a phenomenon that will strongly influence primary productivity and food security under the environmental conditions anticipated during the next few decades". Previously we argued that the weight of evidence in the literature indicated that elevated atmospheric [CO_2] does not inhibit NO_3^- assimilation in C_3 plants (Andrews et al. 2019). New data for common bean (*Phaseolus vulgaris*) and wheat (*Triticum aestivum*) were presented that supported this view and indicated that the effects of elevated atmospheric [CO_2] on nitrogen (N) assimilation and growth of C_3 vascular plants were similar regardless of the form of N assimilated. Bloom et al. (2019) strongly criticised the arguments presented in Andrews et al. (2019). Here we respond to these criticisms and again conclude that the available data indicate that elevated atmospheric [CO_2] does not inhibit NO_3^- assimilation of C_3 plants. Measurement of the partitioning of NO_3^- assimilation between root and shoot of C_3 species under different NO_3^-

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ppl.13096

supply, at ambient and elevated CO_2 would determine if their NO_3^- assimilation is inhibited in shoots but enhanced in roots at elevated atmospheric CO_2.

Introduction

Nitrate (NO_3^-) is likely to be the main form of nitrogen (N) available to, and taken up and assimilated by, most vascular plants in disturbed/ cultivated (well aerated) soils (Andrews et al. 2013; Cameron et al. 2013). Most C_3 vascular plants have the ability to assimilate NO_3^- in their root and shoot with the relative importance of the two parts of the plant dependent on genotype and environmental conditions, in particular, NO_3^- availability (Andrews 1986). For many species, the proportion of total plant NO_3^- assimilation in the shoot increases with increased NO_3^- supply. Within the plant, NO_3^- is reduced to NO_2^- by the enzyme nitrate reductase (NR) and then this NO_2^- is reduced to NH_4^+ by nitrite reductase (NiR). In turn, the NH_4^+ is assimilated into the amino acids glutamine and glutamate via the glutamine synthetase (GS)/ glutamate synthase (GOGAT) pathway (Andrews et al. 2004; Lea and Miflin, 2011; Xu et al. 2012).

Nitrate reductase is located in the cytosol of root and shoot cells and, for most species tested, uses NADH as the reductant for the conversion of NO_3^- to NO_2^- (Xu et al. 2012). The NiR enzyme ($NO_2^- > NH_4^+$) is located in the plastids of roots and other non-photosynthetic tissue, and the chloroplasts of photosynthetic tissue, and uses ferredoxin (Fd) as a reductant (Hanke and Mulo 2013). Within the plastids/ chloroplasts, GS (GS2) catalyses the ATP-dependent conversion of this NH_4^+ and glutamate to glutamine, while GOGAT catalyses the NADH- or Fd- dependent conversion of glutamine and 2-oxoglutarate to form two molecules of glutamate. The NADH-dependent GOGAT is located predominantly in non-photosynthesising cells where reductant for NO_3^- reduction and glutamate synthesis is initially supplied by the oxidative pentose phosphate pathway (Bowsher et al. 2007). The Fd-dependent GOGAT activity is much greater than NADH-GOGAT in shoot/ leaves, where the ATP and reductant for NiR, GS2 and Fd-GOGAT can be derived directly from photosystems I and II in illuminated chloroplasts (Lea and Miflin 2011).

Over several papers, Bloom and co-workers argued that C_3 plants respond more positively to elevated [CO_2] with NH_4^+ (assimilated primarily in roots) than with NO_3^- as N source because elevated CO_2 inhibits their photoreduction of NO_3^- and hence reduces total plant N assimilation and growth (Bloom 2015a,b; Rubio-Asensio and Bloom 2017). They argued that under ambient CO_2, photorespiration stimulates the export of malate from chloroplasts to the cytoplasm and this malate in the cytoplasm generates NADH that drives the reduction of NO_3^- to NO_2^-. Under elevated CO_2, however, photorespiration is inhibited which causes a decrease in transport of malate from the chloroplast to the cytoplasm and consequently decreased generation of NADH and associated NO_3^- assimilation in the cytoplasm. Previously we argued that the weight of evidence in the literature indicates that elevated atmospheric [CO_2] does not inhibit NO_3^- assimilation in C_3 plants (Andrews et al. 2019). Also, new data for common bean (*Phaseolus vulgaris*) and wheat (*Triticum aestivum*) were presented that supported this view and suggested that the effects of elevated atmospheric [CO_2] on N assimilation and growth of C_3 vascular plants will be similar regardless of the form of N assimilated. Bloom et al. (2019) responded to Andrews et al. (2019) and three other papers (Dier et al. 2018; Abadie and Tcherkez 2019; Tcherkez and Limami 2019) that “purport to present counterevidence” to their proposal “that rising atmospheric CO_2 concentrations inhibit malate production in chloroplasts and thus impede assimilation of NO_3^- into protein in shoots of C_3 plants”. Here we focus on their response to Andrews et al. (2019).

Response to Bloom et al. (2019)

Bloom et al. (2019) listed several points in our paper (Andrews et al. 2019) where in their view we made false claims in relation to the literature or misinterpreted it. For example, the first point raised was that our statement “the weight of evidence in the literature indicates that elevated atmospheric [CO_2] does not inhibit NO_3^- assimilation and growth of C_3 vascular plants” consists of four studies that exposed plants to a specific nitrogen form. This is a misinterpretation of our arguments. The weight of evidence in the literature that we referred to included considerable data sets from free air carbon dioxide (FACE) trials carried out under conditions (cultivated/ aerated soils) in which NO_3^- was likely to have been the main form of N available to plants (Andrews et al. 2013, 2019). These studies indicated that a wide range of C_3 species, including wheat, show increased growth under CO_2 enrichment, especially if they receive high applications of N (a selected eight references were given for this point that included meta-analyses and reviews). Continuing, Bloom et al.

(2019) stated that the four studies we highlighted actually support their conclusions: this is not the case. These studies indicated that for wheat (Hocking and Meyer 1991), tobacco (*Nicotiana tabacum*; Geiger et al. 1999; Matt et al. 2001) and cucumber (*Cucumis sativus*; Dong et al., 2017) supplied NO_3^- as the sole N source under controlled environment or glasshouse conditions, greatest growth and reduced N accumulation across treatments occurred under elevated CO_2 with high NO_3^- supply. Considering the first study (Hocking and Meyer 1991), Bloom et al. (2019) argued that wheat with NO_3^- as a sole N source accumulated less reduced N per DW in its shoots under elevated than ambient CO_2 affirming that elevated CO_2 inhibited total NO_3^- assimilation. This is a major point of difference in our views. The results of Hocking and Meyer (1991) for wheat are similar to those presented for wheat and common bean under NO_3^- nutrition in Andrews et al. (2019). Specifically, elevated CO_2 substantially increased growth of common bean and wheat under NO_3^- nutrition. Also, for both species, at limiting and optimal NO_3^- supply, total plant reduced N was greater at elevated than ambient CO_2 indicating that greater NO_3^- assimilation had occurred at elevated CO_2. Nevertheless, the proportional increase in total plant N content was not as great as that for DW and thus tissue N content per unit DW was consequently lower with elevated CO_2. The results of Hocking and Meyer (1991) must be interpreted carefully as elevated CO_2 was only supplied during the day and it could be argued that plants may have increased the proportion of NO_3^- assimilation carried out at night to mitigate CO_2 inhibition of shoot NO_3^- assimilation during the light period. However, elevated CO_2 was maintained over the 24 h in the three other studies highlighted (Geiger et al. 1999; Matt et al. 2001; Dong et al. 2017).

The increase in total plant DW relative to total plant N and the resultant decrease in tissue N per unit DW at elevated CO_2 has been termed 'N dilution' and has been linked to increased accumulation of non-structural carbohydrates and plant secondary compounds (Taub and Wang 2008). In some cases, elevated CO_2 can increase photosynthesis in the short term, but if photosynthate utilisation is inadequate, a source sink imbalance can arise, leading to end-product (carbohydrate) accumulation and subsequent down-regulation of photosynthesis linked to lower ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) concentration and activity (Ainsworth and Rogers 2007; Zheng et al. 2019; Beechey-Gradwell et al. 2020). In their Summary, Bloom et al. (2019) stated that "hundreds of papers" support their proposal that rising atmospheric CO_2 concentrations inhibit malate production in chloroplasts and thus impede assimilation of NO_3^- into protein in shoots of C_3 plants. We strongly disagree with this statement, but many papers do report a decrease in tissue N/ protein content g^{-1} DW with elevated CO_2. We emphasise that the general effects of elevated [CO_2] on growth and N assimilation of wheat with NO_3^- or NH_4^+ as N source, and common bean under NO_3^-, NH_4^+, urea and N_2 fixation nutrition were similar regardless of N form supplied. In all cases, total plant DW and total plant reduced N were greater at elevated [CO_2] but tissue N g^{-1} DW was lower. These results led to our suggestion that the effects of elevated atmospheric CO_2 concentration on N assimilation and growth of C_3 plants will be similar regardless of the form of N assimilated including NO_3^-.

We highlight one other area of our work criticised by Bloom et al. (2019). Bloom et al. (2019) stated that we used shoot nitrate reductase activity (NRA) and shoot organic N concentration as proxy measures for shoot NO_3^- assimilation *in planta* but NRA seldom limits NO_3^- assimilation, and organic N in shoots is derived not only from shoot NO_3^- assimilation but also from import of amino acids generated by NO_3^- assimilation in roots. The main point made in Andrews et al. (2019) relating to the experiments carried out was that for wheat and common bean under low and high NO_3^- supply, total plant reduced N was greater at elevated than ambient CO_2. For both species, the shoot is likely to have been the main site of NO_3^- assimilation at ambient CO_2 (Andrews et al. 1992, 2013). However, we acknowledge that we did not measure the contribution of the root to reduced N in the shoot of either species at ambient or elevated CO_2. Generally, NR is a substrate (NO_3^-) induced enzyme and tissue NRA often correlates with tissue NO_3^- assimilation although we concede it is unlikely to give an accurate measure of its NO_3^- assimilation *in situ* (Andrews et al. 2013; Bloom et al. 2019). In Andrews et al. (2019), leaf NRA for wheat increased with increased NO_3^- supply and the associated increased total plant reduced N at ambient and elevated CO_2. Also, the *in vivo* NRA assay used relies on endogenous NADH to reduce NO_3^- to NO_2^- (NADH is not included in the assay buffer), and thus similar values for lamina NRA at ambient and elevated CO_2 indicate that NADH was not limiting NO_3^- reduction under elevated relative to ambient CO_2. Bloom et al. (2019) stated that exposure to elevated CO_2 atmosphere stimulates root assimilation. Again, we did not determine if this was the case for wheat or common bean in our study. However, in our view, if elevated CO_2 did inhibit shoot NO_3^- assimilation, it seems highly unlikely that for common bean or wheat, a shift from shoot to root NO_3^- assimilation could be great enough to give increased NO_3^-

assimilation per plant with elevated than ambient CO_2 at optimum NO_3^- supply but this needs testing.

The two most common methods used to quantify the partitioning of NO_3^- assimilation between root and shoot are measurement of the relative proportions of total plant NRA in the two plant parts and xylem sap analysis for NO_3^- and reduced N. The proportion of xylem sap N as NO_3^- -N is taken as the proportion of total plant NO_3^- assimilation carried out in the shoot. Data from both sets of measurements must be interpreted carefully. For example, as outlined above, tissue NRA is unlikely to give an accurate measure of NO_3^- assimilation in situ. The main weakness of xylem sap analysis for NO_3^- and reduced N is that it does not indicate the proportion of xylem sap N that is cycling (as organic N) between root and shoot. This can be substantial in some cases. Modelling approaches have been developed to counter possible inaccuracies in determining the partitioning of NO_3^- assimilation between root and shoot from NRA distribution in the plant and xylem sap analysis. Generally, models involve the quantitative measurement of NO_3^- uptake, its movement, storage and assimilation in the different parts of the plant and cycling of reduced N in the phloem and xylem (Jeschke and Pate 1991). We agree with Bloom et al (2019) that for some species, root assimilation increases in importance under elevated CO_2 but data are few and this effect is inconsistent (Table 1). For example, focussing on the studies quoted by Bloom et al. (2019), a quantitative modelling approach indicated that the proportion of total plant NO_3^- assimilation in the shoot decreased from 80% at ambient CO_2 to 57% at elevated CO_2 for *Nicotiana tabacum* supplied 5 mol m^{-3} applied NO_3^- (Kruse et al. 2002). However, for poplar (*Populus tremula* x *P. alba*), a quantitative modelling approach indicated that elevated CO_2 shifted the partitioning of NO_3^- assimilation towards the root at low NO_3^- supply but not at high NO_3^- supply (Kruse et al. 2003). The NRA distribution data of Jauregui at al. (2016) indicated that for Arabidopsis supplied 0.8 mol m^{-3} NO_3^-, almost all NO_3^- was assimilated in the shoot at ambient and elevated CO_2 (Table 1).

Bloom et al. (2019) presented new data on ^{15}N isotope discrimination in wheat and Arabidopsis that they claim show shoot NO_3^- assimilation decreased and root assimilation increased under elevated CO_2, indicating that elevated CO_2 inhibited shoot NO_3^- assimilation while it enhanced root NO_3^- assimilation. Their approach is limited for several reasons. For example, there are no data on the N isotope ratio of the source NO_3^-, so absolute values for discrimination cannot be calculated and in the absence of dry matter data for root and shoot, a mass balance of the isotopes is not possible. The measurements of isotope ratios in the roots and in the shoots include both organic and inorganic N. The extent of discrimination in organic N in the roots depends not only on the root NRA but also on the NO_3^- efflux as a fraction of NO_3^- influx. The shoot delta ^{15}N gives no independent evidence of shoot NO_3^- assimilation into organic matter. Also, a shift in the partitioning of NO_3^- assimilation from shoot to root under elevated CO_2 supply does not confirm inhibition of NO_3^- assimilation in photosynthetic tissue. For example, there is evidence for barley (*Hordeum vulgare*) that reduced levels of reductant (NADH) limit NO_3^- assimilation in roots at high NO_3^- supply (Andrews et al. 1992). Greater root NO_3^- assimilation under elevated CO_2 could be due to greater transport of photosynthate to the root which is utilised in the production of reductant and increased root biomass (Hocking and Meyer 1991; Andrews et al. 2006).

Conclusions and a way forward

In our view, as argued above, the weight of evidence in the literature indicates that elevated atmospheric [CO_2] does not inhibit NO_3^- assimilation in C_3 plants (Andrews et al. 2019). If, as proposed by Bloom et al. (2019), inhibition of photorespiration causes a decrease in transport of malate from the chloroplast to the shoot cytoplasm and consequently decreased generation of NADH in the cytoplasm this does not impact on NO_3^- assimilation at plant level. Indeed, there are reports for several C_3 species that NO_3^- assimilation per plant was greater at elevated CO_2. Nevertheless, total plant DW also increased with elevated CO_2, and the proportional increase in total plant N content was not as great as that for DW thus tissue N content per unit DW was lower with elevated CO_2. Detailed studies of the partitioning of NO_3^- assimilation between root and shoot of a range of C_3 species under different NO_3^- supply, at ambient and elevated CO_2 would be a next step to determine if rising atmospheric CO_2 concentration inhibits NO_3^- assimilation in shoots but enhances it in roots of this group of plants. Arabidopsis should be included in the study to relate to previous work and also to determine how elevated CO_2 affects expression of genes involved in NO_3^- assimilation and associated processes.

Author contributions

MA drafted the manuscript with input from all authors and all authors agreed on the final version of the manuscript.

Acknowledgments – We thank the Editor and the two anonymous referees for their constructive criticism of the original manuscript. The University of Dundee is a registered Scottish Charity, NO 015096

References

Abadie C, Tcherkez G (2019) *In vivo* phosphoenolpyruvate carboxylase activity is controlled by CO_2 and O_2 mole fraction and represents a major flux at high photorespiration rates. New Phytol 221: 1943-1852.

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ 30: 258-270.

Andrews M (1986) The partitioning of nitrate assimilation between root and shoot of higher plants. Plant Cell Environ 9: 511-519.

Andrews M, Condron LM, Kemp PD, Topping JF, Lindsey K, Hodge S, Raven JA (2019) Elevated CO_2 effects on nitrogen assimilation and growth of C_3 vascular plants are similar regardless of N-form assimilated. J Exp Bot 70: 683-690.

Andrews M, Lea PJ, Raven JA, Lindsey K (2004) Can genetic manipulation of plant nitrogen assimilation enzymes result in increased crop yield and greater N-use efficiency? An assessment. Ann Appl Biol 145: 25-40.

Andrews M, Morton JD, Lieffering M, Bisset L (1992) The partitioning of nitrate assimilation between root and shoot of a range of temperate cereals and pasture grasses. Ann Bot 70: 271-276.

Andrews M, Raven JA, Lea PJ (2013) Do plants need nitrate? The mechanisms by which nitrogen form affects plants. Ann Appl Biol 163: 174-199.

Andrews M, Raven JA, Lea PJ, Sprent JI. (2006) A role for shoot protein in shoot-root dry matter allocation in higher plants. Ann Bot 97: 3-10.

Bauer GA, Berntson GM (2001) Ammonium and nitrate acquisition by plants in response to elevated CO_2 concentration: the roles of root physiology and architecture. Tree Physiol 21: 137-144.

Beechey-Gradwell Z, Cooney L, Winichayakal S, Andrews M, Hea SY, Crowther T, Roberts N (2020) Storing carbon in leaf sinks enhances perennial ryegrass carbon capture especially under high N and elevated CO_2. J Exp Bot doi: 10. 1093/jxb/erz494.

Bloom AJ (2015a) Photorespiration and nitrate assimilation: a major intersection between plant carbon and nitrogen. Photosynth Res 123: 117-128.

Bloom AJ (2015b) The increasing importance of distinguishing among plant nitrogen sources. Curr Opin Plant Biol 25: 10-16.

Bloom AJ, Kasemsap P, Rubio-Asensio JS (2019) Rising atmospheric CO_2 concentration inhibits nitrate assimilation in shoots but enhances it in roots of C_3 plants. Physiol Plant: doi: 10.1111/ppl.13040.

Bowsher CG, Lacey AE, Hanke GT, Clarkson DT, Saker LR, Stulen I, Emes MJ (2007) The effect of G1c6P uptake and its subsequent oxidation within pea root plastids on nitrite reduction and glutamate synthesis. J Exp Bot 58: 1109-1118.

Cameron KC, Di HJ, Moir JL (2013) Nitrogen losses from the soil/ plant system: a review. Ann Appl Biol 162: 145-173.

Dier M, Meinen R, Erbs M, Kollhorst L, Baillie C-K, Kaufholdt D, Kücke M, Weigel H-J, Zörb C, Hänsch R, Manderscheid R (2018) Effects of free air carbon dioxide enrichment (FACE) on nitrogen assimilation and growth of winter wheat under nitrate and ammonium fertilization. Global Change Biol 24: e40-e54.

Dong J, Li X, Chu W, Duan Z (2017) High nitrate supply promotes nitrate assimilation and alleviates photosynthetic acclimation of cucumber plants under elevated CO_2. Sci Hortic 218: 275-283.

Geiger M, Haake V, Ludewig F, Sonnewald U, Stitt M (1999) The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. Plant Cell Environ 22: 1177-1199.

Hanke G, Mulo P (2013) Plant type ferredoxins and ferredoxin-dependent metabolism. Plant Cell Environ 36: 1071-1084.

Hocking PJ, Meyer CP (1991) Effects of CO_2 enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. Aust J Plant Physiol 18: 339-356.

Jauregui I, Aparicio-Tejo PM, Avila C, Cañas R, Sakalauskiene S, Aranjuelo I (2016) Root-shoot interactions explain the reduction of leaf mineral content in *Arabidopsis* plants grown under elevated [CO_2] conditions. Physiol Plant 158: 65-79.

Jeschke WD, Pate JS (1991) Modelling of the partitioning, assimilation and storage of nitrate within root and shoot organs of castor bean (*Ricinus communis* L.). J Exp Bot 42: 1091-1103.

Kruse J, Hetzger I, Hänsch R, Mendel R-R, Walch-Liu P, Engels C, Rennenberg H (2002) Elevated pCO_2 favours nitrate reduction in the roots of wild-type tobacco (*Nicotiana tabacum* cv. Gat.) and significantly alters N-metabolism in transformants lacking functional nitrate reductase in the roots. J Exp Bot 53: 2351-2367.

Kruse J, Hetzger I, Mai C, Polle A, Rennenberg H (2003) Elevated pCO_2 affects N-metabolism of young poplar plants (*Populus tremula* x *P. alba*) differently at deficient and sufficient N-supply. New Phytol 157: 65-81.

Lea PJ, Miflin BJ (2011) Nitrogen assimilation and its relevance to crop improvement. In: Foyer CH, Zhang H (eds) Annual Plant Reviews, Nitrogen Metabolism in Plants in the Post-Genomic Era, Vol 42. Chichester, UK: Wiley-Blackwell. Chichester, pp 1-40.

Matt P, Geiger M, Walch-Liu P, Engels A, Krapp A, Stitt M (2001) Elevated carbon dioxide increases nitrate uptake and nitrate reductase activity when tobacco is growing on nitrate, but increases ammonium uptake and inhibits nitrate reductase activity when tobacco is growing on ammonium nitrate. Plant Cell Environ 24: 1119-1137.

Rubio-Asensio JS, Bloom AJ (2017) Inorganic nitrogen form: a major player in wheat and Arabidopsis responses to elevated CO_2. J Exp Bot 68: 2611-2625.

Taub DR, Wang X (2008) Why are nitrogen concentrations in plant tissues lower under elevated CO_2? A critical examination of the hypotheses. J Integr Plant Biol 50: 1365-1374.

Tcherkez G, Limami AM (2019) Net photosynthetic CO_2 assimilation: more than just CO_2 and O_2 reduction cycles. New Phytol 223: 520-529.

Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency. Ann Rev Plant Biol 63: 153-182.

Zheng Y, Li F, Hao L. Yu J, Guo L. Zhou H, Ma C, Zhang X, Xu M (2019) Elevated CO_2 concentration induces photosynthetic down-regulation with changes in leaf structure, non-structural carbohydrates and nitrogen content of soybean. BMC Plant Biol 19: 255.

Table 1. Collated values for the effect of elevated atmospheric [CO_2] on the proportion of nitrate (NO_3^-) assimilation carried out in the shoot of vascular plant species as indicated by the distribution of nitrate reductase activity (NRA) between the two plant parts, xylem sap analysis (XSA) for NO_3^- and reduced N or a quantitative model (M).

Species	% NO_3^- assimilation in shoot (NO_3^- supply)		Method used	Comments	References
	(≤1 mol m^{-3})	(>1.0 mol m^{-3})			
Arabidopsis thaliana	96 (0.8) 97 (0.8)		NRA	Unaffected by elevated [CO_2]	Jauregui et al. (2016)
Betula alleghaniensis		49 (1.6) 76 (1.6)	NRA	Decreased at elevated [CO_2]	Bauer and Berntson (2001)
Nicotiana tabacum		75 (5) 78 (5)	XSA	Unaffected by elevated [CO_2]	Kruse et al. (2002)
		57 (5) 80 (5)	M	Decreased at elevated [CO_2]	
	88 (1) 89 (1)	89 (2) 89 (2)	NRA	Unaffected by elevated [CO_2]	Matt et al. (2001)
Pinus stroba		3 (1.6) 10 (1.6)	NRA	Unaffected by elevated [CO_2]	Bauer and Berntson (2001)
Populus tremula × P. alba	4 (0.2) 30 (0.2)	44 (2) 47 (2)	XSA/M	Decreased at elevated [CO_2] at low but not high NO^- supply	Kruse et al. (2003)