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Review

^{15}N methodologies for quantifying the response of N_2-fixing associations to elevated [CO_2]: A review

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ABSTRACT

Methodologies based on ^{15}N enrichment (*E*) and ^{15}N natural abundance (*NA*) have been used to obtain quantitative estimates of the response of biological N_2 fixation (BNF) of legumes (woody, grain and forage) and actinorhizal plants grown in artificial media or in soil exposed to elevated atmospheric concentrations of carbon dioxide e[CO_2] for extended periods of time, in growth rooms, greenhouses, open top chambers or free-air CO_2 enrichment (FACE) facilities. $^{15}N_2$ has also been used to quantify the response of endophytic and free-living diazotrophs to e[CO_2]. The primary criterion of response was the proportional dependence of the N_2-fixing system on the atmosphere as a source of N. i.e. the symbiotic dependence (P_{atm}). The unique feature of ^{15}N-based methods is their ability to provide time-integrated and yield-independent estimates of P_{atm}. In studies conducted in artificial media or in soil using the *E* methodology there was either no response or a positive response of P_{atm} to e[CO_2]. The interpretation of results obtained in artificial media or with $^{15}N_2$ is straight forward, not being subject to the assumptions on which the *E* and *NA* soil-cultured methods are based. A variety of methods have been used to estimate isotopic fractionation attendant on the *NA* technique, the so-called 'B value', which attaches a degree of uncertainty to the results obtained. Using the *NA* technique, a suite of responses of P_{atm} to e[CO_2] has been published, from positive to neutral to sometimes negative effects. Several factors which interact with the response of N_2-fixing species to e[CO_2] were identified.

Contents

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1. Introduction

The response of components of terrestrial ecosystems to increasing concentrations of atmospheric carbon dioxide [CO_2] has been a scientific line of enquiry since the 1960s. Initially, growth chamber experiments were conducted but were then followed by open top chambers and free-air CO_2 enrichment (FACE) facilities. Contributions of biological N_2 fixation (BNF) via free-living, endophytic or symbiotic micro-organisms are collectively an important source of N addition to natural and agricultural ecosystems. It is therefore important to understand if BNF may be affected by increasing atmospheric [CO_2], and in which way and by how much. Early studies relied on the use of the acetylene reduction assay or other biochemical markers to obtain qualitative estimates of the response of BNF to [CO_2], but such methods are clearly inadequate and quantitative estimates are needed.

The quantitative estimation of the proportional contribution of N_2 fixation to the N nutrition of soil-cultured legumes or other N_2-fixing associations (i.e. the symbiotic dependence, P_{atm}) is accomplished by the application of ^{15}N methodologies which are either direct ($^{15}N_2$) or indirect (^{15}N dilution), the latter generally being based either on artificial ^{15}N enrichment or ^{15}N natural abundance (Chalk, 2016; Chalk et al., 2016). For the estimation of the amount of N_2 fixed (i.e. the symbiotic performance), the N yield (i.e. dry matter yield × N concentration) is multiplied by P_{atm}. In order to separate the effect of any given variable on symbiotic performance, both N yield and P_{atm} must be determined independently, which is the unique strength of the ^{15}N methodologies. Several studies have demonstrated that P_{atm} is more resilient to stress factors such as nutrient deficiencies (Chalk, 2000), sodicity (Smith et al., 2009) or drought (Chalk et al., 2010) compared to N yield, and that the stress must be acute before P_{atm} is significantly reduced.

A general review of the literature on the effect of elevated [CO_2] (e[CO_2]) on legume BNF was provided by Rogers et al. (2009). A meta-analysis of published data on estimates of the effect of e[CO_2] on P_{atm} and N yield of grain and pasture legumes was published by Lam et al. (2012b). From a data set of 27 observations from 9 studies it was concluded that there was a 38% increase in the yield of fixed N and a 10% (non-significant) increase in P_{atm} due to e[CO_2] from a range of 550 to 730 μmol mol^{-1}, indicating that P_{atm} was much less responsive compared with N yield. The objective of the present review is to revisit this subject by casting a wider net for published ^{15}N-based yield-independent data on legume response to e[CO_2], including not only grain and forage legumes, but also woody legumes, and to extend the coverage further to include actinorhizal, endophytic and free-living associations. Attention will be focused on the correct applications of ^{15}N-based technologies. The aim is to gain an overall quantitative assessment of the effect of e[CO_2] on symbiotic dependence and symbiotic performance and to consider a range of factors which may play an interacting role. We shall not attempt to provide an assessment of the physiological basis for the observed responses or lack thereof.

2. The quantitative response of N_2-fixing plants to e[CO_2]: ^{15}N methodologies

2.1. CO_2 enrichment techniques

The three most widely used CO_2 enrichment techniques are glasshouses/growth chambers, open top chambers (OTC) and free-air CO_2 enrichment (FACE) facilities. While economically practical for a [CO_2] controlled environment, growth chambers are generally limited in scale to accommodate pots or soil cores. Open top chambers (OTC) can house larger scale experiments in the field, but natural wind flow is prevented and the microenvironment is altered by the chamber. Free-air CO_2 enrichment (FACE) facilities are generally favored for larger scale field studies, and although expensive to operate continually there is no perturbation of microclimate within. Several reviews have been written about [CO_2] enrichment facilities, one of the most recent being Uprety et al. (2006). The concentration of CO_2 [CO_2] has been expressed in several units. The standard unit is μmol mol^{-1} which is equivalent to μl l^{-1} or ppm. If expressed as a partial pressure (pCO_2) in Pa, then 1 Pa = 10 μbar = 9.869 μmol mol^{-1}. Henceforth, a[CO_2] will denote the ambient concentration of CO_2 while e[CO_2] will denote the elevated concentration of CO_2.

2.2. Literature search

To assess the effect of e[CO_2] on BNF, we performed extensive keyword searches of several databases (Web of Science, Scopus, CAB Abstracts, Academic Search Complete and Google Scholar) for studies published prior to March 2016. The keywords used in the search included elevated CO_2, (biological) N_2 fixation, ^{15}N, ^{15}N natural abundance, ^{15}N dilution, ^{15}N enrichment, legumes, and their combinations. The search resulted in 26 studies (Tables 1–6).

2.3. Plant culture in artificial media

2.3.1. ^{15}N enrichment (E)

The effect of e[CO_2] on N_2 fixation by plant-microbial associations has been studied by growing the plant under partially-controlled environmental conditions in an artificial rooting medium such as hydroponics or sand watered with ^{15}N-enriched nutrient solution. In this case P_{atm} is estimated according to Eq. (1).

$$P_{atm} = 1 - \frac{E_{plant}}{E_{solution}} \quad (1)$$

Where E is the excess atom fraction ^{15}N. E_{plant} is the difference in the ^{15}N abundance (atom fraction ^{15}N) of the plant in the ^{15}N-enriched treatment minus the ^{15}N abundance of the plant in a control (*NA*) treatment. $E_{solution}$ is the ^{15}N abundance of the solution minus the ^{15}N natural abundance of air (0.003663 atom fraction ^{15}N).

The value of P_{atm} calculated according to Eq. (1) was adjusted by Zanetti et al. (1998) to compensate for the N yield of the plants at age 6 weeks (t_0) before the imposition of the e[CO_2] treatment for a further 36 days (t_1). The adjusted P_{atm} (Eq. (2)) is thus yield dependent.

$$P_{atm}(\text{adjusted}) = \frac{(\text{N yield}_{t1} \times P_{atm}) - \text{N yield}_{t0}}{\text{N yield}_{t1} - \text{N yield}_{t0}} \quad (2)$$

2.3.2. ^{15}N natural abundance (NA)

The artificial rooting medium may also contain plant-available N close to ^{15}N natural abundance. In this case, the N_2-fixing plant is grown in the solution, but in addition it must be grown in an N-free solution where the plant is wholly dependent on N_2 fixation, in order to determine the 'B value'. B is the isotopic fractionation associated with