Soil Biology & Biochemistry 96 (2016) 137–144

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Digging in the dirt – Inadequacy of belowground plant biomass quantification

Anke Hupe [a, *], Hannes Schulz [b], Christian Bruns [b], Rainer Georg Joergensen [a], Florian Wichern [c]

[a] Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany
[b] Organic Farming and Cropping, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany
[c] Faculty of Life Sciences, Rhine-Waal University of Applied Sciences, Marie-Curie-Straße 1, 47533 Kleve, Germany

ARTICLE INFO

Article history:
Received 28 August 2015
Received in revised form
5 November 2015
Accepted 24 January 2016
Available online 8 February 2016

Keywords:
Belowground plant biomass
Mass balance approach
^{15}N
Pea
Rhizodeposition

ABSTRACT

Accurate quantification of belowground plant biomass (BGP) is crucial to account for the carbon (C) and nitrogen (N) stored by plants. As soil sieving to recover roots leaves a large proportion of root borne compounds defined as rhizodeposits (fine roots, root fragments, exudates) unaccounted for, isotope-labelling approaches have frequently been used. The aim of the present study was to compare two approaches that estimate BGP-N from isotope labelling experiments and assess their potential error. *Pisum sativum* was grown in a pot experiment and repeatedly pulse labelled with a ^{13}C glucose and ^{15}N urea solution using a cotton wick method. Additionally, data from a previous study using the same labelling approach were used for comparative BGP-N calculations. In both experiments, the amount of BGP-N calculated with a mass balance approach was significantly lower compared with the classical calculation, indicating substantial overestimation of N rhizodeposition in previous studies. Multiple pulse labelling of plants with ^{15}N can result in homogeneous label distribution, which allows both calculation approaches to be used. However, when label distribution is heterogeneous, the classical approach overestimates N rhizodeposition and BGP-N.

1. Introduction

Soils are one of the major terrestrial carbon (C) sinks and are highly relevant for future C sequestration. It's important to reduce further greenhouse gas emissions from soils (Rees et al., 2005; Lal et al., 2007). Plants act as the major gateway of C input into soils. In particular the belowground plant biomass (BGP), often referred to as "the hidden half" (Den Herder et al., 2010; De Coninck et al., 2015), substantially contributes to C storage of plants and thus to sequestered C (Rees et al., 2005). When estimating nitrogen (N) fixation by legumes, it is important to consider the N stored belowground to obtain accurate values for the N input (Herridge et al., 2008; Peoples et al., 2009). Moreover, an increase in drought periods also in humid regions require adapted cropping systems with plants having extended root systems able to withdraw sufficient quantities of water and making more efficient use of plant nutrients (Franco et al., 2011).

When aiming at quantifying C or nutrients (e.g. N) stored in plants, an accurate estimation of BGP is therefore essential. However, often only parts of the BGP are measured. Most methods for the estimation of root dry matter, such as root sieving, underestimate the extent of the root system and its biomass (BGP), as fine roots are often not determined because of their small size and near transparency (Pierret et al., 2005). Moreover, root exudates and compounds released from dying roots are often not measured. This fraction often unaccounted for when considering visible roots only, can be summarized as rhizodeposits and is measured as C or N released from roots. Consequently, when assessing e.g. BGP-N, beside N stored in roots, all other N derived from roots is defined as N derived from rhizodeposition (NdfR), the process of N release from living plant roots (Uren, 2001; Wichern et al., 2008; Jones et al., 2009; Fustec et al., 2010). The same also holds true for C to some extent, even though other compounds, such as sugars for example, substantially contribute to C rhizodeposition (Nguyen, 2003; Wichern et al., 2008). Therefore, the question arises of how to calculate BGP, in particular rhizodeposition, correctly? Estimating the net rhizodeposition (release minus re-uptake), regardless of

* Corresponding author. Tel.: +49 5542 98 1598; fax: +49 5542 98 1568.
E-mail address: a.hupe@uni-kassel.de (A. Hupe).

http://dx.doi.org/10.1016/j.soilbio.2016.01.014
0038-0717/

whether C or N rhizodeposition, is a complex task and results acquired are influenced by various aspects, such as plant species and age, soil type, nutrient availability in soil, water regime, temperature and pot size (Kuzyakov, 2002; Wichern et al., 2008; Jones et al., 2009; Poorter et al., 2012). However, apart from pot size, the most important factor affecting the prediction of rhizodeposition is the definition used. We therefore (Wichern et al., 2008) propose to use the definition of rhizodeposition given by Uren (2001), who defined all compounds released from roots of living plants as rhizodeposition, including volatiles, gaseous compounds, root fragments and decaying roots.

Beside the definition of rhizodeposition, the method used for labelling plants with stable or radioactive isotopes also fundamentally influences the amount of rhizodeposition predicted, which was shown by Yasmin et al. (2006) and Mahieu et al. (2009). Yasmin et al. (2006) compared leaf, petiole and stem feeding methods for labelling the plants with ^{15}N and observed ^{15}N enrichment and ^{15}N recovery between the treatments, resulting in a high ^{15}N enrichment with leaf feeding and a very low (but more homogeneous) ^{15}N enrichment with petiole feeding. The methods of labelling differ also in the time of solution uptake (Yasmin et al., 2006) and influence the continuity of labelling. Apart from the split root technique, the labelling of the atmosphere with ^{15}N (as Janzen and Bruinsma (1989) did in their work) is a very effective method for a continuous labelling, which is an important assumption for the calculation of rhizodeposition. Due to a multiple pulse labelling, the cotton wick method approached an almost continuous labelling. However, leaf feeding, a method with a fast and effective solution uptake, is only suitable for pulse labelling. Due to this fact, leaf feeding cannot achieve a continuous labelling. Labelling the atmosphere and a multiple pulse labelling are the two most effective methods for estimating a realistic amount of BGP-N, as extensively discussed by Wichern et al. (2008).

Assessing belowground plant N (BGP-N), consisting of root N and rhizodeposition N, is particularly important for total N balances (Arcand et al., 2013a). Janzen and Bruinsma (1989) developed an approach for the calculation of N rhizodeposition after labelling plants with ^{15}N. This approach has been used in most studies quantifying BGP-N inputs used until now. Their approach requires homogeneous distribution of ^{15}N in roots and rhizodeposits in time and space to prevent any dilution of isotopes. However, N is highly relocated within plants during growth (Salon et al., 2001); homogeneous enrichment with ^{15}N is difficult to reach. Tracer relocation may lead to an overestimation of N rhizodeposition because of tracer dilution in roots compared with rhizodeposits or to an underestimation when tracer is accumulated in roots and not released as rhizodeposits (Rasmussen, 2011). The influence of relocation can be limited by a continuous plant labelling until harvest, which is difficult to achieve with the available methods, especially under field conditions (Wichern et al., 2008). Khan et al. (2002) used an isotope mass balance approach to determine BGP-N (^{15}N recovered in roots and soil) as proportion of total N (^{15}N recovered in roots, soil and shoot). With a further development of this mass balance approach, BGP-N and -C could be calculated in mg plant^{-1}. The calculation of BGP-N/BGP-C with an isotope mass balance approach may prevent over- or underestimations caused by tracer relocation processes (Rasmussen, 2011). Therefore, the objectives of the present study were:

- to compare a mass balance approach and the classical approach of Janzen and Bruinsma (1989) for quantification of BGP-N in peas,
- to assess the error associated with the classical calculation when plants are not continuously labelled, and
- to calculate the most realistic amount of BGP-N.

2. Materials and methods

2.1. Soil

For the pot experiment, the soil, collected at 0–30 cm depth from the research station of the University of Kassel in Neu-Eichenberg, Germany (51° 23′ N, 9° 55′ E, 220 m asl), was sieved (10 mm) and stored for six weeks before the experiment started. The silty loam (13% clay; 83% silt; 3% sand) was classified as a Haplic Luvisol (FAO classification, 2014), with a pH of 6.0, 12 mg organic C g^{-1} soil and 1.3 mg total N g^{-1}. At 0–30 cm soil depth, the soil contained 17 μg Ca-acetate lactate extractable P g^{-1} soil, 66 μg K g^{-1} soil, and 84 μg Mg g^{-1} soil. One day before sowing, the soil was fertilized with 100 kg Ca ha^{-1}. Fertilizers (N, P, K, and Mg) were added at the day of sowing (150/17/43/9 kg ha^{-1}). The soil used in the column experiment was silty loam (16–23% clay, 75–82% silt, 2% sand) from the research station of the University of Kassel, which is located at Frankenhausen, Northern Hesse, Germany (51° 24′ N, 9° 25′ W, 230 m asl). For more information see Wichern et al. (2007a).

2.2. Experimental designs and labelling

There were two experimental designs, one for *Pisum sativum* cv. Frisson (in 2012) and one for *P. sativum* cv. Santana (in 2005). Frisson was cultivated under controlled conditions in the greenhouse with a relative humidity of 60% and 110 klxh d^{-1}. The mean temperature was 20 °C during the day and 15 °C during the night. The 8.5 l pots (28 cm diameter and 20 cm height) were filled with 11 kg soil and mechanically compressed to a wet density of 1.3 g cm^{-3}. Two plants per pot were cultivated with six replicates per treatment. For mycorrhization, all pots were inoculated with "rootgrowth™ professional", placed directly below the seed. Once a week, the pots were weighed and watered with deionized water, to keep a WHC between 60 and 80%. For Santana, the collected columns were placed in a box filled with soil and 4 plants per column were cultivated. For more details see Wichern et al. (2007a).

All plants (Santana and Frisson) were labelled with a ^{15}N urea (95 atom%) and ^{13}C glucose (99 atom%) solution, using a stem feeding technique (Russell and Fillery, 1996; Wichern et al., 2007b). The stem of each plant was drilled with a 0.5 mm drill, approximately 3 cm above ground. Then, a cotton wick was passed through the hole. The ends of the wick were passed through silicon tubes and through the lid of a two ml vial, which contained the labelling solution. To prevent evaporation losses, the connections between wick and plant and between wick and lid were sealed with Teroson (Henkel), a kneading mass. The feeding solution was produced with deionized water and then sterile filtrated (<0.2 μm). All material used for labelling was steam sterilized for 20 min at 121 °C. Frisson was labelled fortnightly with a 0.5% urea and 2% glucose solution. For labelling, the plants were multiple pulse labelled (5 times overall), beginning at BBCH 13 (3 leaves unfolded) 14 days after sowing (DAS) (Lancashire et al., 1991). The concentration of urea and glucose differed for Santana, depending on the estimated dry matter increase (between 0.6% and 8.1% for glucose and between 0.04% and 0.89% for urea). Plants were labelled twice with 1 ml solution, first in BBCH 15 (5 leaves unfolded; 33 DAS) and then in BBCH 18 (8 leaves unfolded; 45 DAS). After solution uptake, the empty vials of Frisson and Santana were filled with deionized water (between 0.5 ml and 1 ml) to secure a complete solution uptake. Pots and columns were covered with a 1 mm mesh to prevent soil contamination from falling leaves.