

# A simple methodology to estimate plant volume in nitrous oxide emission studies

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## Abstract

Closed-chamber methodology is widely used for the estimation of greenhouse gas (GHG) emissions in agricultural systems. The volume displaced by plants inside chambers influences GHG flux estimation, although generally it is not discounted from chamber headspace in the calculation. A novel image analysis-based procedure is proposed to estimate plant volume and to assess its impact on nitrous oxide (N<sub>2</sub>O) flux estimations in a wheat (*Triticum aestivum* L. ‘Rimbaud’) crop. A maximum of 2.2% of the 13-L chambers was displaced by plants, leading to a systematic 0.9% overestimation in cumulative N<sub>2</sub>O emissions if plant volume was not considered. Thus, plant canopy volume should be taken into account for improving the accuracy of emissions.

## 1 | INTRODUCTION

Due to climate change concerns, the number of scientific publications related to greenhouse gas (GHG) emissions from agricultural systems has increased exponentially in recent years (Parkin, Venterea, & Hargreaves, 2012). Although a variety of techniques are available for GHG measurement (Holland, Robertson, Greenberg, Groffman, & Boone, 1999) and several recent reviews have made methodological recommendations (De Klein & Harvey, 2015; Olf et al., 2018; Pavelka et al., 2018), there is no standard methodology for flux measurements. Most flux measurement studies are performed using chamber-based techniques, whereby gas samples are collected and subjected to infrared or gas chromatography analysis (Eugster & Merbold, 2015). Plant volume inside chambers is rarely, if ever, measured and discounted from chamber headspace in the GHG flux calculation (Morton & Heinemeyer, 2018) despite the fact that plant volume reduces the effective chamber headspace and leads to inaccurate flux estimations (Livingston, Hutchinson, Matson, & Harriss, 1995). As a consequence of disregarding plant volume, an overestimation of the fluxes is expected (Morton & Heinemeyer, 2018).

In this context, the objectives of the current study were (a) to propose and evaluate a new image analysis-based procedure to estimate plant volume inside closed chambers, (b) to assess the proportion of chamber displaced by wheat canopy at different stages using the image-based procedure, and (c) to determine the error associated with disregarding plant volume on nitrous oxide (N<sub>2</sub>O) emissions.

**Abbreviations:** GHG, greenhouse gas.

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## 2 | MATERIALS AND METHODS

Irrigated bread wheat (*Triticum aestivum* L. ‘Rimbaud’) was grown (2016–2017) in a deep silty-loam textured soil classified as Typic Xerofluvent (Soil Survey Staff, 2014). The experimental design was a randomized block with four treatments and four replicates. The treatments included a non-N-fertilized control and three pig slurry treatments with different additives at the same target rate (120 kg NH<sub>4</sub><sup>+</sup>-N ha<sup>-1</sup>). Sixteen plots (2.0 m × 3.6 m) configured the trial; each had one static closed unvented chamber for GHG measurement. To meet the distinct objectives of this study, the experimental design as described was used as a framework for collecting plant volume and N<sub>2</sub>O data.

The closed-chamber technique and the N<sub>2</sub>O flux measurement procedures were the same as those described by Mateo-Marín, Quílez, Guillén, and Isla (2020). Briefly, a collar (0.30 m i.d., 0.12 m height) was inserted 0.10 m into the soil. At the time of flux measurements, an upper cover of 0.165-m height was located on top of each collar, creating a 13.1-L headspace volume. The height of the upper cover did not change during the course of the study; plants were folded when necessary to facilitate chamber closure. This strategy did not affect plants’ growth because of their flexibility, although some stems were damaged on the last sampling date just before harvest. Inner air samples (15 ml) were drawn at 0 and 60 min after chamber closure using a polypropylene syringe and injected into 12-ml Exetainer borosilicate pre-evacuated glass vials (Labco Ltd.). Chambers were sampled on 12 dates between 7 Apr. and 20 June 2017; samplings occurred daily for the first 5 d after fertilization (7 Apr. 2017) and decreased in the frequency afterward. Air samples were analyzed by gas chromatography with an Agilent 7890B equipped with an electron capture detector for determining N<sub>2</sub>O concentration. The N<sub>2</sub>O flux was estimated as the difference between the final and initial N<sub>2</sub>O concentrations (corrected by air temperature) divided by the time interval between the two sampling times and multiplied by the ratio between the headspace and the area of soil covered by the chamber (MacKenzie, Fan, & Cadrin, 1998).

A novel, nondestructive procedure is proposed to estimate the volume displaced by the plants inside the chambers. The approach is based on the relationship between canopy image area (derived from zenithal images) and plant volume. Wheat plants located inside the collars were described periodically according to their phenological stage (Zadoks, Chang, & Konzak, 1974) and photographed. At the same time, in an area adjacent to the experimental plots, a secondary chamber collar was established to photograph wheat plants encompassed by it at the same phenological stage. All plants inside this secondary collar (0.071 m<sup>2</sup>) were cut, frozen (−30 °C), and placed into a glass test tube to determine their volume by water displacement. Three differently sized test tubes (500,

### Core Ideas

- A method using zenithal images was proposed to estimate plant volume accurately.
- Estimated canopy wheat area was strongly related to plant volume ( $R^2 = .96$ ).
- Maximum plant volume relative to chamber headspace (2.2%) was reached at anthesis.
- N<sub>2</sub>O emissions were overestimated by 0.9% when plant volume was not considered.

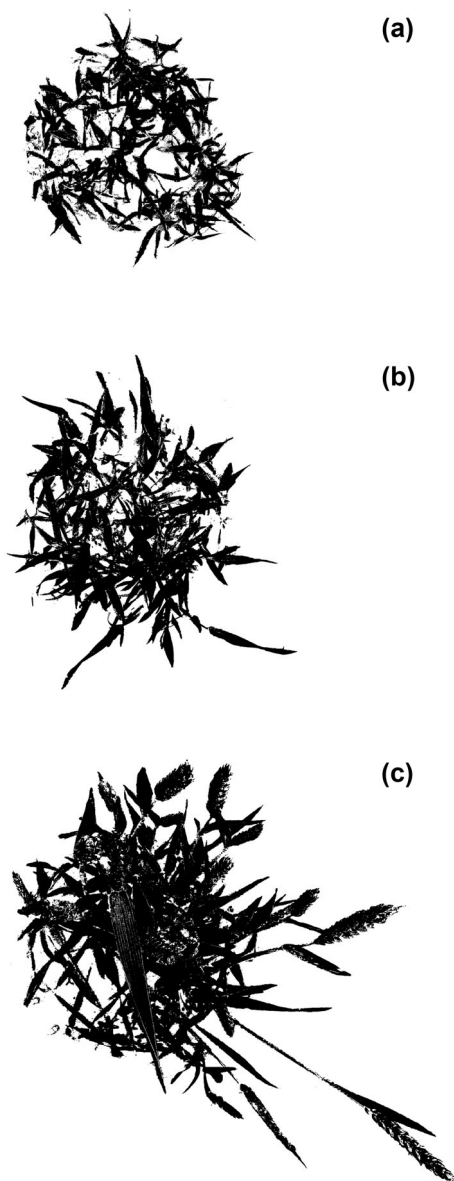
1,000, and 2,000 ml) were used throughout the trial, with sequentially larger tubes used as plant volumes expanded due to growth. Between two and six measurements were used at each phenological stage of plants to determine canopy image area and plant volume.

Zenithal photographs were managed according to the orthoimage technique for canopy image analysis described by Lordan et al. (2015) to obtain the area projected by the canopy. Photographs were taken ( $2.3 \times 10^3$  pixels cm<sup>-2</sup>) with a compact camera (Canon PowerShot SX210 IS) at 1.20-m height over the soil surface. Plants outside the collar were covered (hidden) by a piece of cardboard to isolate all the canopy area projected outside the vertical projection of the collar. A ruler was added on the piece of cardboard to scale the image. The photographed green area was isolated (Photoshop CS5, Adobe Systems) and processed using ImageJ (Rasband, 1997–2018) to select all the wheat canopy pixels, obtaining the canopy image area (Figure 1), which was corrected by the image scale. The relation between plant volume and canopy image area was established using a linear regression model that pooled data from all phenological stages. Then, the volume of the plants within each collar located in the experimental plots was estimated from their canopy image area by using the linear model and solving for plant volume.

## 3 | RESULTS

Wheat plant volume can be precisely estimated through canopy image analysis using the equation presented in Figure 2, where there was a strong relationship between the two variables ( $R^2 = .96$ ;  $p < .001$ ; RMSE, 18.2 ml). The measured volume of the plants located inside the collar ranged from 0.6 to 2.2% of the chamber volume (CV, 1–11%) depending on the phenological stage. The maximum plant volume (2.2%) was measured at anthesis (stage 65 according to the Zadoks scale) (Figure 3).

When the N<sub>2</sub>O emissions (Figure 4) were calculated by adjusting for the proportion of the chamber displaced

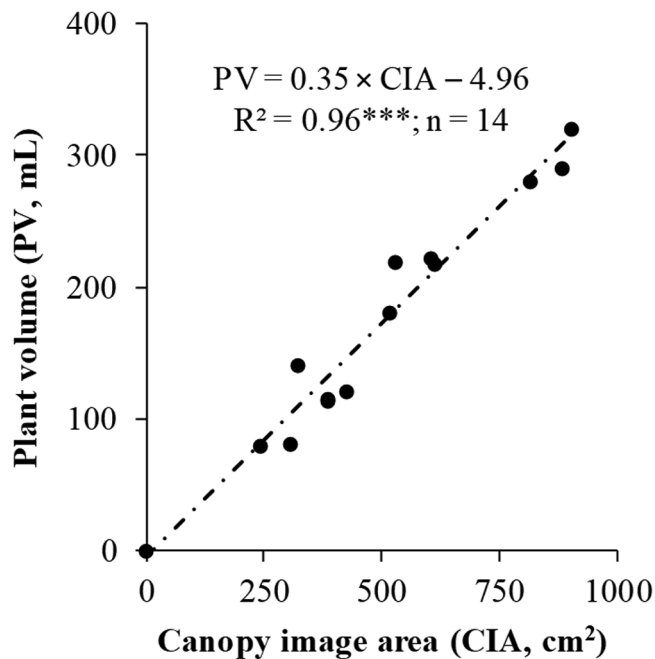


**FIGURE 1** Isolation and selection of green area corresponding to wheat located within a chamber at Zadoks scale stage (a) 32 (second node detectable), (b) 45 (boots swollen), and (c) 65 (anthesis half way)

by wheat plants (thereby changing the chamber headspace volume), the cumulative  $N_2O$  emissions were 0.9% lower ( $646.7 \text{ g N ha}^{-1}$  vs.  $652.5 \text{ g N ha}^{-1}$ ; mean difference,  $5.8 \pm 0.5 \text{ g N ha}^{-1}$ ) than when plant volume was disregarded from the calculations.

#### 4 | DISCUSSION

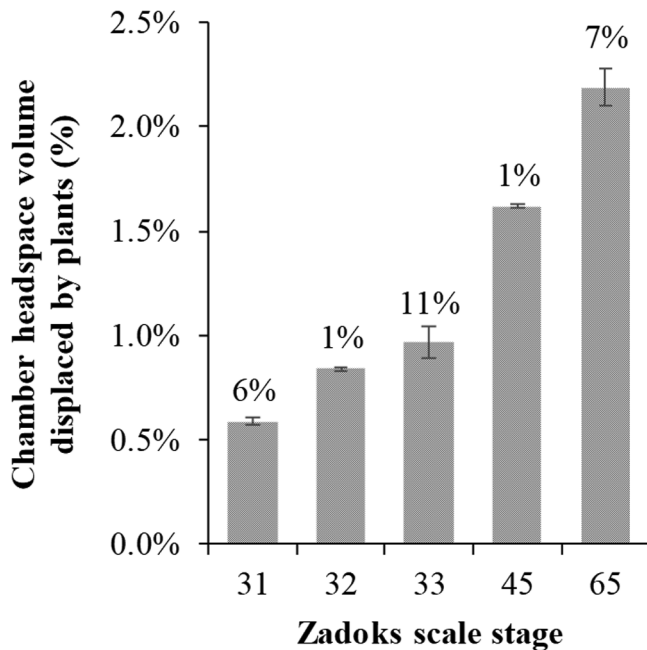
The image analysis proposed here is a viable methodology to adjust for changes in headspace volume due to plant growth inside chambers. There was a small error in plant volume estimation and a high correlation between the estimated canopy



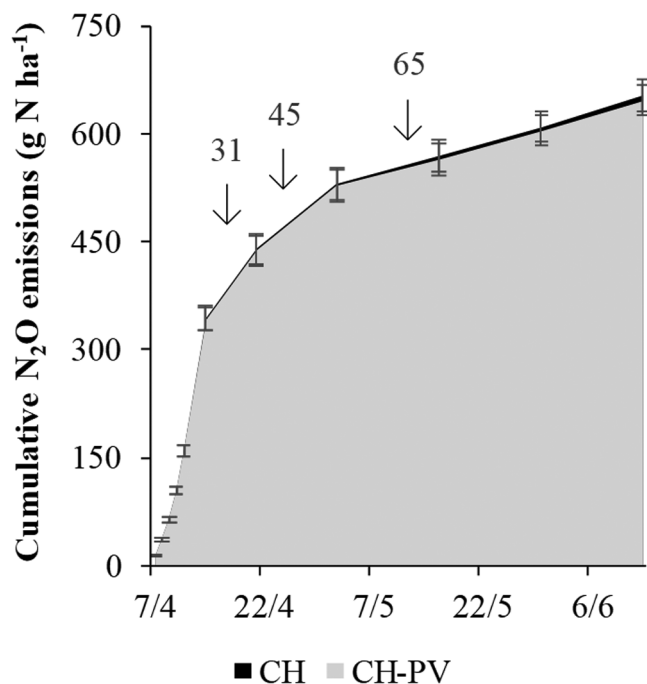
**FIGURE 2** Relationship between wheat canopy image area and plant volume

image area and the measured volume of plants. This image-based method fulfills the premises of Morton and Heinemeyer (2018) regarding the necessity of a simple, effective, and non-destructive method for assessing plant volume in chamber-based techniques for GHG measurements. In addition, it is a more objective methodology than the visual assessment of two observers proposed by Morton and Heinemeyer (2018). It is advisable to establish a relationship between plant volume and canopy image area for each experiment, even for crops similar to the one in this study, because differences in plant architecture are expected among cultivars with different growth habits. The determination of plant volumes by the water displacement method using test tubes could present a challenge when whole plants do not fit into test tubes, but it could be solved by breaking up the plants prior to freezing.

According to the results, cumulative  $N_2O$  emissions were slightly overestimated when disregarding plant volume in the calculations, which was a negligible but systematic error. The smaller contribution of plant volume to differences in cumulative  $N_2O$  emissions (0.9%) compared with the volume of chamber displaced by plants (0.6–2.2%) was a result of plant volume being low when emissions were at their greatest. Similar results were observed by Collier, Dean, Oates, Ruark, and Jackson (2016), who detected small but significant effects on calculated fluxes after adjusting for 1.4–2.2% the within-chamber alfalfa volume (variation of 0.7–1.7% in the flux rate). Disregarding plant volume may be more relevant for long-term experiments and for emission factor estimation because plant volume is lower in unfertilized than in fertil-



**FIGURE 3** Mean volume of the chamber displaced by wheat plants at different growth stages. Vertical lines show SE; numbers above the bars indicate CV. Zadoks scale stage: 31, first node detectable; 32, second node detectable; 33, third node detectable; 45, boots swollen; 65, anthesis half way



**FIGURE 4** Cumulative  $N_2O$  emissions with time whether plant volume was not discounted from the chamber headspace (CH) and whether plant volume was discounted (CH-PV) for calculation of emissions. Arrows indicate the Zadoks scale stage (31, first node detectable; 45, boots swollen; 65, anthesis half way) at three moments. Vertical lines show SE

ized plots. Therefore, in agreement with Collier et al. (2016), estimating plant volumes whenever possible is recommended. Nonetheless, researchers' objectives (e.g., to obtain emission factors, compare different treatments, quantify absolute emission values) will dictate the relevance of considering the plant volume into the calculations.

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#### CONFLICT OF INTEREST

There are no conflicts of interest.

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