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_2O emissions were overestimated by 0.9% when plant volume was not considered.

A Simple Methodology to Estimate Plant Volume in Nitrous Oxide Emission Studies

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Abbreviations

GHG: greenhouse gas.

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_2O) flux estimations in a wheat 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_2O emissions if plant volume was not considered. Thus, plant canopy volume should be taken into account for improving the accuracy of emissions.

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 et al., 2012). A variety of techniques are available for GHG measurement (Holland et al., 1999) and several recent reviews have made methodological recommendations (De Klein and Harvey, 2015; Olfs et al., 2018; Pavelka et al., 2018), but there is no standard methodology for flux measurements. Most flux measurement studies are performed using chamber-based techniques where gas samples are collected and subjected to infrared or gas chromatograph analysis (Eugster and Merbold, 2015). Plant volume inside chambers is rarely, if ever, measured and discounted from chamber headspace in the GHG flux calculation (Morton and Heinemeyer, 2018), despite the fact that plant volume reduces the effective chamber headspace and leads to inaccurate flux estimations (Livingston et al., 1995). As a consequence of disregarding plant volume, an overestimation of the fluxes is expected (Morton and Heinemeyer, 2018).

In this context, the objectives of the current study were (1) to propose and evaluate a new image analysis-based procedure to estimate plant volume inside closed chambers, (2) to assess the proportion of chamber displaced by wheat canopy at different stages using the image-based procedure, and (3) to determine the error associated with disregarding plant volume on nitrous oxide (N_2O) emissions.

MATERIALS AND METHODS

Irrigated bread wheat (*Triticum aestivum* L. cv. '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 randomised block with four treatments and four replicates. The treatments included a non-N fertilised control and three pig slurry

59 treatments with different additives at the same target rate (120 kg NH_4^+-N ha^{-1}). Sixteen
60 plots (2.0 m × 3.6 m) configured the trial; each had one static closed unvented chamber
61 for GHG measurement. The experimental design as described was used as a framework
62 for collecting plant volume and N_2O data, to meet the distinct objectives of this study.

63 The closed-chamber technique and the N_2O flux measurement procedures were
64 the same than those described by Mateo-Marín et al. (2020). Briefly, a collar (0.30 m
65 inner diameter and 0.12 m height) was inserted 0.10 m into the soil. At the time of flux
66 measurements, an upper cover of 0.165-m height was located on top of each collar,
67 creating a 13.1-L headspace volume. The height of the upper cover did not change during
68 the course of the study; plants were folded when necessary to facilitate chamber closure.
69 This strategy did not affect plants' growth because of their flexibility, although at the last
70 sampling date just before harvest, some stems were damaged. Inner air samples (15 mL)
71 were drawn at 0 and 60 minutes after chamber closure using a polypropylene syringe and
72 injected into 12-mL Exetainer® borosilicate pre-evacuated glass vials (Labco Ltd.,
73 Lampeter, UK). Chambers were sampled on 12 dates between 7 Apr. and 20 June 2017:
74 samplings occurred daily for the first 5 days after fertilisation (7 Apr. 2017) and decreased
75 in the frequency afterwards. Air samples were analysed by gas chromatography with an
76 Agilent 7890B equipped with an electron capture detector for determining N_2O
77 concentration. The N_2O flux was estimated as the difference between the final and initial
78 N_2O concentrations (corrected by air temperature), divided by the time interval between
79 the two sampling times and multiplied by the ratio between the headspace and the area of
80 soil covered by the chamber (MacKenzie et al., 1998).

81 A novel non-destructive procedure is proposed to estimate the volume displaced
82 by the plants inside the chambers. The approach is based on the relationship between the
83 canopy image area (derived from zenithal images) and the plant volume. Wheat plants

84 located inside the collars were described periodically according to their phenological
85 stage (Zadoks et al., 1974) and photographed. At the same time, in an area adjacent to the
86 experimental plots, a secondary chamber collar was established to photograph wheat
87 plants encompassed by it at the same phenological stage. All plants inside this secondary
88 collar (0.071 m^2) were cut, frozen (-30 ºC), and placed into a glass test tube to determine
89 their volume by water displacement. Three differently sized test tubes (500 mL, 1,000
90 mL, and 2,000 mL) were used throughout the trial, with sequentially larger tubes used as
91 plant volumes expanded due to growth. Between two and six measurements were used at
92 each phenological stage of plants to determine canopy image area and plant volume.
93 Zenithal photographs were managed according to the orthoimage technique for
94 canopy image analysis described by Lordan et al. (2015) in order to obtain the area
95 projected by the canopy. Photographs were taken ($2.3 \cdot 10^3$ pixels cm^{-2}) with a compact
96 camera (Canon PowerShot SX210 IS) at 1.20-m height over the soil surface. Plants
97 outside the collar were covered (hidden) by a piece of cardboard to isolate all the canopy
98 area projected outside the vertical projection of the collar. A ruler was added on the piece
99 of cardboard to scale the image. The photographed green area was isolated (Photoshop
100 CS5, Adobe Systems) and processed using ImageJ (Rasband, 1997–2018) to select all the
101 wheat canopy pixels, obtaining the canopy image area (Fig. 1), which was corrected by
102 the image scale. The relation between plant volume and canopy image area was
103 established using a linear regression model pooling data from all phenological stages.
104 Then, the volume of the plants within each collar located in the experimental plots was
105 estimated from their canopy image area by using the linear model and solving for plant
106 volume.
107

RESULTS

Wheat plant volume can be precisely estimated through canopy image analysis using the equation presented in Fig. 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 from 1 to 11%) depending on the phenological stage. The maximum plant volume (2.2%) was measured at anthesis (stage 65 according to the Zadoks scale; Fig. 3).

When the N_2O emissions (Fig. 4) were calculated by adjusting for the proportion of the chamber displaced by wheat plants (thereby changing the chamber headspace volume), the cumulative N_2O emissions were 0.9% lower (646.7 g N ha^{-1} vs. 652.5 g N ha^{-1}; mean difference 5.8 ± 0.5 g N ha^{-1}) than when plant volume was disregarded from the calculations.

DISCUSSION

The image analysis proposed here is a viable methodology to adjust for changes in headspace volume due to plant growth inside chambers, as there was a small error in plant volume estimation and a high correlation between the estimated canopy image area and the measured volume of plants. This image-based method fulfils the premises of Morton and Heinemeyer (2018) about 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, since 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, namely a negligible but systematic error. The smaller contribution of plant volume to differences in cumulative N_2O emissions (0.9%) compared to 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 et al. (2016), who detected small although significant effects on calculated fluxes after adjusting for 1.4-2.2% the alfalfa volume within-chamber (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 since plant volume is lower in unfertilised than in fertilised plots. Therefore, in agreement with Collier et al. (2016), it is recommended estimating plant volumes whenever possible. 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.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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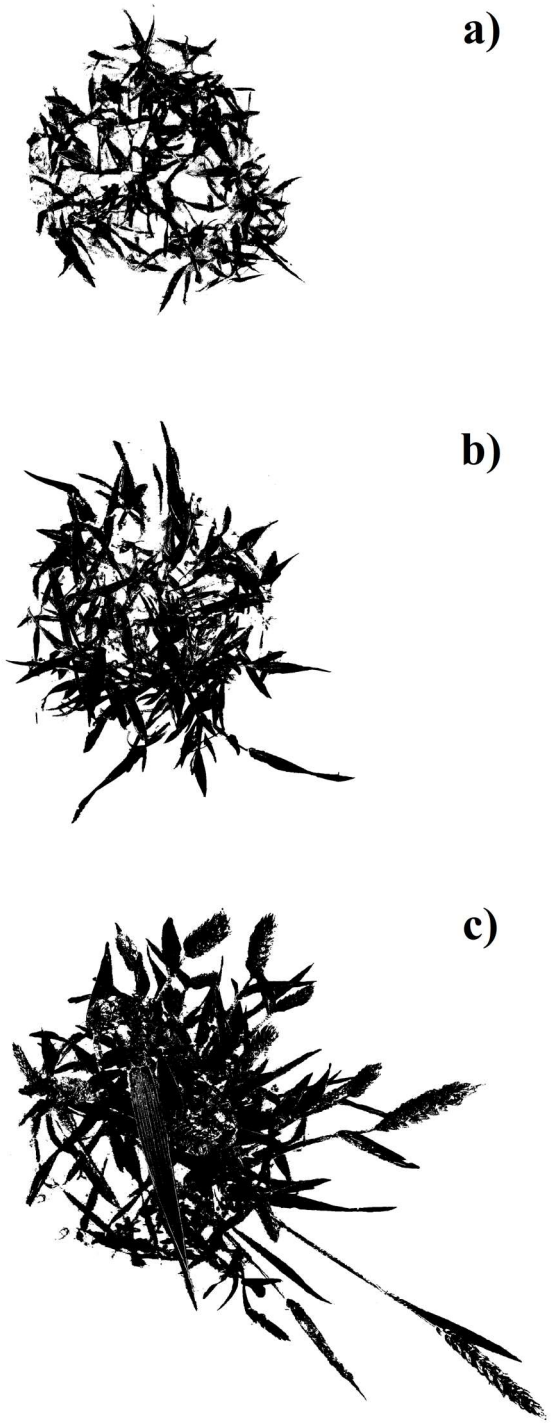
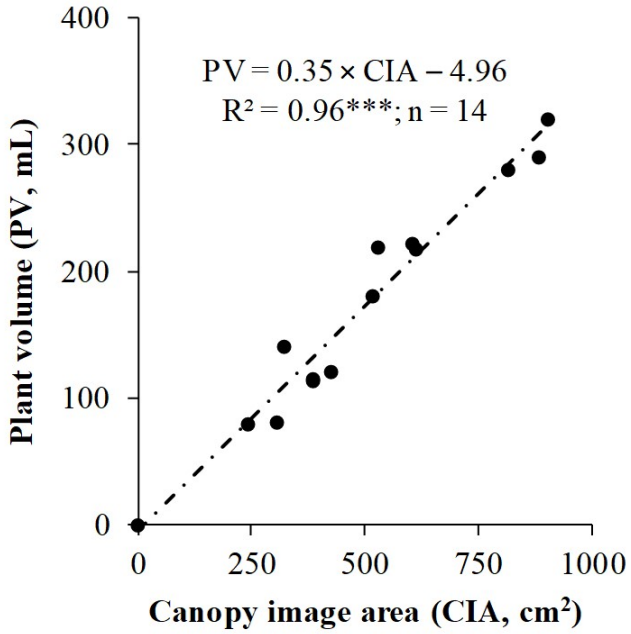
205 **FIGURES**



206

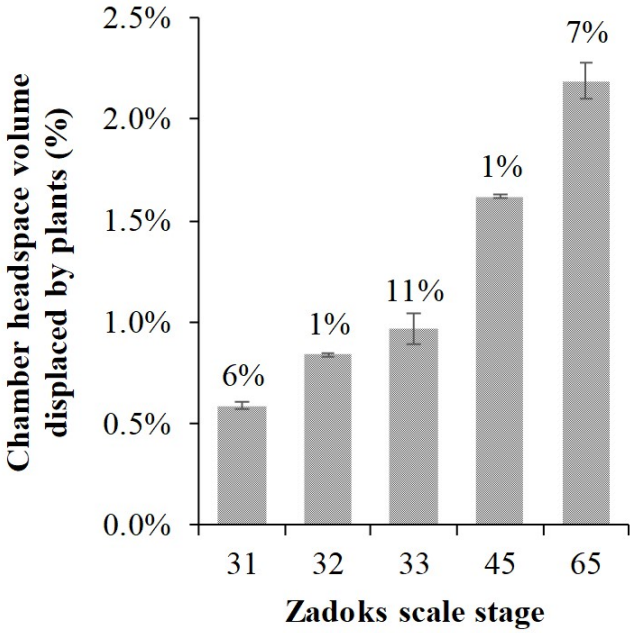
207 **Figure 1.** Isolation and selection of green area corresponding to wheat located within a
208 chamber at Zadoks scale stages 32 (2nd node detectable, Fig. 1a), 45 (Boots swollen, Fig.
209 1b), and 65 (Anthesis half-way, Fig. 1c).

210



211

212 **Figure 2.** Relationship between the wheat canopy image area (cm^2) and the plant volume
213 (PV, mL).
214



215

216 **Figure 3.** Mean volume (%) of the chamber displaced by wheat plants at different growth
217 stages*. Vertical lines show the standard error and numbers above the bars indicate the
218 coefficient of variation.

219 *Zadoks scale stage: 31- 1st node detectable, 32- 2nd node detectable, 33- 3rd node detectable, 45- Boots
220 swollen, and 65. Anthesis half-way.
221

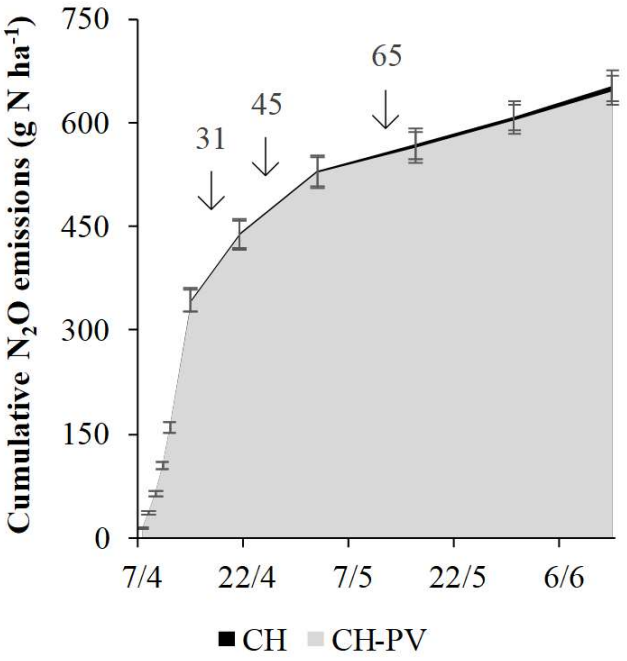


Figure 4. Cumulative N_2O emissions with time (g N ha^{-1}) whether plant volume was not discounted from the chamber headspace (CH) and whether plant volume was discounted (CH-PV) for the calculation of the emissions. Arrows indicate the Zadoks scale stage (31- 1st node detectable, 45- Boots swollen, and 65. Anthesis half-way) at three moments. Vertical lines show the standard error.