A novel technique to label cover crop biomass using stable isotopes

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Abstract

Stable isotopes can be used as tracers for carbon and nitrogen pathways being a great tool to track nutrients in integrated systems. The objective of this experiment was to understand the partitioning of ^{15}N and ^{13}C within cover crop plants when they were labeled with stable isotopes, using chambers under field conditions. Cover crops were planted at the University of Florida, North Florida Research and Education Center-Marianna, located in Marianna, FL. Treatments were four cover crops, in which one was considered a typical cover crop system and the other three consisted of an integrated crop-livestock system with or without the inclusion of legume or different nitrogen fertilizer rates grazed every two weeks. All treatments were replicated three times in a randomized complete block design. Two chambers were built and placed in each plot to label the cover crop plants. For the ^{15}N labeling, $^{15}N_2$-labeled urea (98 atom% ^{15}N) was applied at a rate of 0.5 kg N ha^{-1} only once. The target amount of $^{13}CO_2$ (99 atom% ^{13}C) was determined considering a 20% enrichment of the CO_2 concentration present inside the chamber's volume. The $^{13}CO_2$ labeling was performed for 28 consecutive days. The labeling technique using chambers and stable isotopes to enrich cover crop species worked under field conditions for both, grass and legume species. Moving forward, this labeling technique can be a useful tool to track nutrient pathways, especially litter decomposition in diversified integrated crop and livestock systems under different management practices.

Introduction

Incorporation of cover crops into cropping systems have many benefits such as reduction of soil erosion, increase in soil organic matter, increase in soil microbial activity, and overall soil health, especially when integrating diversified crop–livestock systems (Franzluebbers and Stuedemann, 2014; Farmaha et al., 2021). Stable isotopes can be used as tracers for carbon and nitrogen pathways being a great tool to track nutrients in integrated systems (Fry, 2006). The objective of this experiment was to understand the partitioning of ^{15}N and ^{13}C within cover crop plants when they were labeled with carbon and nitrogen stable isotopes, using chambers under field conditions.

Methods

Cover crops were planted in plots that measured 7.3 x 15.2 m at University of Florida, North Florida Research and Education Center-Marianna, located in Marianna, FL (30°52'36.0"N 85°11'20.0"W). Treatments were four cover crops, in which one was considered a typical cover crop system and the other three consisted of an integrated crop-livestock system with or without inclusion of legume or different nitrogen fertilizer rates grazed every two weeks (Table 1). All treatments were replicated three times in a randomized complete block design.

Table 1. Treatments description.

Treatment	Description
CC[a]	Row crop, oat cover crop and no grazed
ICL+L[b]	ICL: Row crop, oat/crimson cover crop. Grazed every two weeks
ICL+LN[c]	ICL: Row crop, oat/crimson cover crop + 34 kg N ha^{-1}. Grazed every two weeks
ICL+N[d]	ICL: Row crop, oat cover crop + 90 kg N ha^{-1}. Grazed every two weeks

[a] CC, conventional cover crop; [b] ICL+L, integrated crop-livestock system with inclusion of legume; [c] ICL+L, integrated crop and livestock system with inclusion of legume and nitrogen fertilizer; [d]ICL+N integrated crop and livestock system and nitrogen fertilizer.

Two chambers measuring 0.56 x 0.56 x 0.68-m were built and placed in each plot to label the cover crop plants. A 2-mm transparent acrylic sheet held by a wooden structure was used as wall and roof to make sure sun light was still reaching the plants whenever the chambers were in use. An 8.5-cm height wood base was built and placed 5-cm into the soil three days before the labeling started. A 1.9-cm window seal foam was sticked to the bottom of the chamber to guarantee a complete seal when in contact with the base and to avoid any air leak from the bottom of the chamber. On the acrylic roof, a 1-cm diameter circle was drilled to place a septum for the $^{13}CO_2$ gas injection. Waterproof silicon sealant was then used around the corners of the acrylic sheets and around the septum to avoid air leak.

For the ^{15}N labeling, $^{15}N_2$-labeled urea (98 atom% ^{15}N) was applied at a rate of 0.5 kg N ha^{-1} only once on March 24th, 2022, considering the base area. The $^{13}CO_2$ labeling, on the other hand, was performed for 28 consecutive days, one hour after sunrise from March 25th to April 21st, 2022. The target amount of $^{13}CO_2$ (99 atom% ^{13}C) was determined considering a 20% enrichment of the CO_2 concentration present inside the chamber's volume. The labeled gas was injected into the chamber through the septum at once and chambers remained closed until temperature inside reached 28 °C to avoid triggering photorespiration (Paulsen, 1994). Four 473-ml cups full of ice were placed in each chamber to help the temperature remain below 28°C. A battery fan was also placed inside the chamber to allow for the labeled $^{13}CO_2$ circulate homogeneously (Figure 1).

At the end of the labeling period, plants were then harvested at ground level, dried at 55°C for 72 h. In the plots containing grass and legume mixture, botanical composition was performed. In both species, plant was then separated in different fractions: steam, leaf, senescent material (leaves), and inflorescence. Subsamples from the different fractions of both species were then ground to pass a 2-mm screen using a Wiley Mill (Model 4, Thomas-Wiley laboratory Mill, Thomas Scientific), ball-milled in a Mixer Mill MM 400 (Retsch) for nine min at 25 Hz and analyzed for $\delta^{15}N$ and $\delta^{13}C$ using a CHNS analyzer (Vario Micro Cube, Elementar) coupled to an isotope ratio mass spectrometer (IsoPrime).

Output variables were analyzed using the PROC GLIMMIX from SAS (SAS/STAT 15.1, SAS Institute). Treatments and fractions were considered fixed effect and blocks were considered random effect.

Figure 1. Chambers used for $^{13}CO_2$ cover crops labeling in North Florida.

Results and Discussion

The labeling technique using chambers and stable isotopes to enrich cover crop species worked under field condition for both, grass, and legume species. The different fractions for both plants also presented more enriched (e.g., more positive) values for $\delta^{15}N$ and $\delta^{13}C$ overall (Figures 2 and 3). There was no fraction effect for $\delta^{15}N$ on the grass ($P = 0.32$). The $\delta^{15}N$ averaged 577‰ across the entire plant components. On the other hand, the legume presented a fraction effect ($P = 0.02$) for $\delta^{15}N$ in which senescent material, leaves, and steams had greater values for $\delta^{15}N$ (199.45, 166, and 150.88‰) when compared to the inflorescence (106.64‰).

A fraction effect was observed for both grass ($P < 0.0001$) and legume ($P < 0.0001$) species when looking at the $\delta^{13}C$ values. Senescent material presented less positive $\delta^{13}C$ values for both species when compared to the other fractions of the plant. In the grass, inflorescence showed a greater $\delta^{13}C$ enrichment (97.91‰), followed by the leaves and stems (54.03, 69.85 ‰); however, for the legume, inflorescence and stems had more positive $\delta^{13}C$ values (72.32, 71.56 ‰), followed by the leaves (50.20‰). It is likely that these $\delta^{13}C$ values represent a carbohydrate shift followed by plants when there is a need for -reallocating reserves when it is time to invest in flower and seed development (Vaillant-Gaveau et al., 2011). The sink-source theory could also be an explanation, since the inflorescence starts being a new sink for carbohydrates and old leaves undergo a transition from a carbon sink to a carbon source; such transition in forages is marked by cessation of carbohydrates going into mature leaves and occur later in the development of the forage plants (Skinner and Moore, 2007).

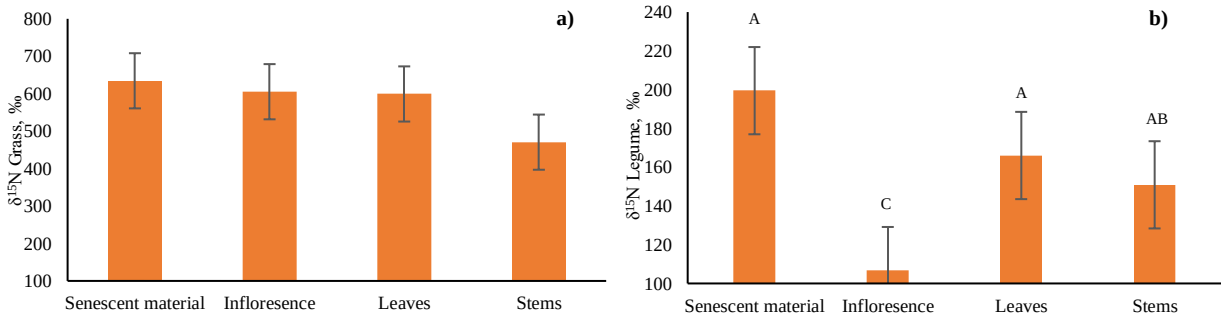

Figure 2. a) No differences ($P = 0.32$) for $\delta^{15}N$ on the grass different fractions. b) Fraction difference ($P = 0.02$) $\delta^{15}N$ on the different fraction of the legume.

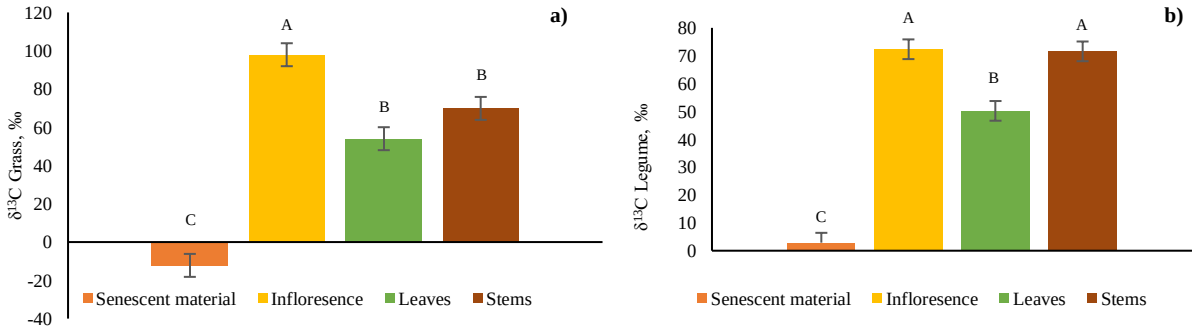

Figure 3. a) Fraction differences for $\delta^{13}C$ ($P < 0.0001$) on the different fraction of the grass. b) Fraction differences for $\delta^{13}C$ ($P < 0.0001$) on the different fraction of the legume. ($P < 0.0001$).

Conclusions and Implications

The labeling technique using chambers and stable isotopes to enrich cover crop worked under field condition using chambers for both, grass, and legume species. Moving forward, this labeling technique can be a useful tool to track nutrient pathways, especially litter decomposition and greenhouse gas emissions from the soil in diversified integrated crop-livestock systems under different management practices.

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