



Contribution to N₂O emissions and N fate of crop residues and synthetic N fertilizer in a cover crop–maize rotation: A ¹⁵N-tracing study

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Abstract

Background and aims Crop residue retention and cover cropping are key strategies in conservation agriculture, but their effects on nitrogen (N) cycling and nitrous oxide (N₂O) emissions, particularly in semiarid croplands, remain underexplored. This study aimed to evaluate the N dynamics and N₂O sources in an irrigated cover crop–maize rotation in central Spain by conducting a ¹⁵N tracing experiment.

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Methods ¹⁵N-labeled maize (*Zea mays* L.) residues were incorporated into the soil, followed by vetch (*Vicia faba* L.), barley (*Hordeum vulgare* L.) cover crops, or bare fallow. A subsequent maize crop was fertilized with 210 kg N ha⁻¹ as ammonium nitrate (AN) or ¹⁵N-labeled AN (¹⁵NH₄NO₃, ¹⁵AN//NH₄¹⁵NO₃, A¹⁵N) in the microplots receiving ¹⁵N-enriched or unlabeled maize residues, respectively.

Results Cumulative N₂O emissions were 47% higher under vetch than under barley. Average N₂O emission factors were 0.05% for ¹⁵N-labeled residues and 0.23% for fertilizer. Most N₂O originated from ¹⁵AN (42%–61%), followed by endogenous sources (i.e., mineralization of organic N from soil or crop residues, 19%–42%) and A¹⁵N (9%–18%). Maize recovered 7.7% of residue N, with no differences between cover cropping treatments. Plant uptake from A¹⁵N was 2.3 times higher than from ¹⁵AN. Across barley, vetch, and fallow, total N recovery averaged 33% in plants and 22% in soil. Barley cover cropping improved fertilizer N recovery in maize by 57% (*P* < 0.05) compared with fallow.

Conclusion These findings highlight the role of cereal cover crops and the regulation of ammonium oxidation-based processes in enhancing N use efficiency and reducing fertilizer-derived N₂O emissions in semiarid agro-ecosystems.

Keywords ^{15}N isotope dilution technique · Conservation agriculture · Green manure · Semiarid croplands · N recovery efficiency · Mediterranean climate

Introduction

Crop residue retention provides a substantial input of organically bound nitrogen (N) and carbon (C), thereby enhancing soil fertility and increasing soil organic C (SOC) stocks (Palm et al. 2014; Peng et al. 2023). Soil organic C contents increase by 2.7%–18.2% when residues are incorporated into the soil versus when they are removed (Bolinder et al. 2020). These inputs are especially important in semiarid regions, where soils typically have low organic C concentrations, with implications for soil chemical, physical, and biological quality. However, synchronizing N release from organic sources with crop demand is a key challenge for sustainable agriculture, as poor timing may reduce yields and N use efficiency (NUE). Mineral N availability is primarily regulated by microbial processes—mineralization and immobilization—which depend on N source composition, environmental factors (e.g., moisture and temperature), and soil properties (Elrys et al. 2021; Wang et al. 2024; Jiang et al. 2025).

Crop residue retention also influences other key N-cycling processes, leading to reactive N losses, including nitrous oxide (N_2O) emission to the atmosphere (IPCC 2022). Biochemical processes leading to N_2O emissions, mainly nitrification and denitrification, are driven by changes in the physical and chemical properties of the soil, which are in turn influenced by management practices such as crop rotation and cover cropping (Ussiri and Lal 2013). Understanding and quantifying these processes regulating N_2O emissions is essential to design effective mitigation strategies. A meta-analysis by Abalos et al. (2022b) found that when crop residues are incorporated into the soil, the N supplied to soil microorganisms results in an average 40%–50% increase in N_2O emissions compared with when residues are removed. However, this effect is strongly dependent on the quality of the residues, specifically their C:N ratio (Chen et al. 2013; Hu et al. 2019; Abalos et al. 2022a). In general, the incorporation of mature crop residues with a C:N ratio of > 30 (such as those from maize stover)

stimulates microbial immobilization by N released during residue degradation in the soil, thereby lowering N_2O emissions (Li et al. 2016; Abalos et al. 2022b). In contrast, N-rich crop residues (i.e., those with a low C:N ratio), such as green manures, provide an energy (degradable organic C) and mineral N substrate source for nitrifying and denitrifying microorganisms, which can increase N_2O emissions in the soil (Li et al. 2016), mainly during the residue decomposition period (Basche et al. 2014). These authors found a sixfold increase of N_2O emissions due to cover crop decomposition, which was higher than the cover crop induced-increment during the cash crop period (i.e., 117%). The C:N ratio is indeed strongly and positively correlated with the maturity index, encompassing both soluble (i.e., associated with higher N_2O fluxes, such as water-extractable carbon and, soluble Van Soest fiber and N content) and structural fractions (i.e., associated with lower N_2O fluxes, such as the contents of cellulose, hemicellulose, and lignin) that influence residue decomposition rates and C:N release (Abalos et al. 2022b). When these residues come from cover crops, their effect during and after the cover cropping period also depends on other factors, such as rhizodeposition, organic C input, and residual N left after N uptake by cover crops, which is enhanced in nonlegume species (Guardia et al. 2025). Moreover, intensified annual rotations that include both cover crops and cash crops involve the co-existence of residues with different maturity and composition, which hampers accurate quantification of N_2O fluxes and the attribution of each N input's contribution, including potential synergistic/antagonistic effects on biochemical N-cycling processes.

Including cover crops in annual irrigated rotations as an alternative to periods of bare fallow can enhance agro-ecosystem sustainability by reducing N leaching (a major N loss pathway) and increasing soil N retention (Quemada et al. 2013; Gabriel et al. 2013; Abdalla et al. 2019; Li et al. 2021). The incorporation of N-rich green manure residues before maize cropping also reduces the need for mineral N fertilizer for the main crop, thus potentially reducing synthetic N fertilizer needs and therefore upstream emissions as a key component of the C footprint in semiarid croplands (Guardia et al. 2019; Aguilera et al. 2021). Guardia et al. (2016) found that barley rather than vetch cover crop residues stimulated N_2O emissions from N fertilizer (i.e., 150% increment), but without

distinguishing between denitrification and ammonium (NH_4^+ -N) oxidation-based processes. Indeed, these processes were shown to be dominant even in semiarid irrigated crops (Vallejo et al. 2014; Guardia et al. 2018), where soil frequently reaches moisture saturation conditions, but these are interspersed with drying episodes due to extreme summer temperatures. However, these previous experiments did not include cover crops alternatives during the fallow season and did not address the potential influence of maize residues on N cycling and N_2O emissions in the following cropping phases.

By using ^{15}N tracing techniques, the sources of N_2O emissions (García-Gutiérrez et al. 2023; Guardia et al. 2018) discriminate between different N_2O production processes, such as nitrification and denitrification (Ibraim et al. 2019, 2020). This approach allows for accurate calculation of N_2O emission factors (EFs), N fate (Ding et al. 2019), and crop N recovery efficiency (Yan et al. 2020). Despite numerous studies on ^{15}N recovery from synthetic fertilizer in cash crops (e.g., Gardner and Drinkwater 2009; Yan et al. 2020) or the ^{15}N recovery of N released by cover crops (e.g., Farzadfar and Congreves 2022; Wang et al. 2024; Xu et al. 2025), specific field data on the effect of the preceding cover crop (legume versus nonlegume) on the recovery of synthetic N applied to the cash crop are lacking. The contribution of recalcitrant residues from previous cash crops to N cycling and gaseous N emissions in subsequent rotations remains poorly quantified.

The main objectives of this study were to evaluate the combined effects of incorporating maize residues with two different cover crop species (one legume and one nonlegume) compared with bare fallow on (i) the biochemical processes responsible for N_2O emissions and (ii) the fate of N applied using ^{15}N -labeled maize residues and a ^{15}N -labeled fertilizer. Such scientific objectives represent a potential contribution to the current knowledge because of the complexity of the annual cropping system with three N inputs incorporated at distinct times: autumn (maize stover), spring (cover crop residues), and summer (synthetic N fertilizer). We hypothesized that (i) using a green manure would increase N_2O emissions through substrate addition to the endogenous soil N pool and by priming the mineralization of previous mature maize residues; (ii) the high C:N ratio maize residue would have a limited impact on N_2O emissions and N

cycling during the subsequent cropping phases; and (iii) despite irrigated conditions, nitrification would be a relevant process influencing N_2O emissions from the synthetic fertilizer application.

Materials and methods

Experimental location

The experimental site was located at the National Center of Irrigation and Technology “CENTER” ($40^\circ 25' 1''\text{N}$, $3^\circ 29' 45''\text{W}$) in southern Madrid, Spain. The soil is a *Typic Xerofluvent* with a silt loam texture (10% clay, 59.5% silt, and 30.5% sand), containing 1.2% oxidizable organic C, 9.9% CaCO_3 , 1.6 g total N kg^{-1} , a pH of 8.2, and an EC of 0.5 mS cm^{-1} in the 0–20 cm topsoil. The area is characterized by a semi-arid Mediterranean climate. The mean air temperature and accumulated rainfall over the last 15 years were 14.2 °C and 401 mm, respectively. The climatological data were collected by a meteorological field station located at the CENTER and were uploaded to the “Sistema de Información Agroclimática para el Regadío” (SIAR) webpage (<https://portal.mapa.gob.es/websiar/SeleccionParametrosMap.aspx?dst=1>).

Experimental design

Agricultural practices in the experimental area

The field experiment was conducted from November 2020 to November 2021. The experimental area was divided into three blocks that were randomly split into two plots. Maize residues from the previous cropping season were either incorporated into the topsoil to a depth of 10–15 cm using a shallow disk harrow or removed from the topsoil. In mid-November 2020, the remaining residues were deeply incorporated into the soil to a depth of 40 cm. The three subplots were randomly arranged with barley (*Hordeum vulgare* L. cv. “RGT PLANET”), vetch (*Vicia sativa* L. cv. “Rada”), and bare fallow as cover crops. Cover crops were sown at a rate of 200 kg ha^{-1} on November 17, 2020. They were chemically terminated on April 13, 2021, using a herbicide (Roundup®, glyphosate 36%, 5 L ha^{-1}) and then incorporated into the soil with a disk harrow at a depth of 10–15 cm on May 6, 2021. Maize (*Zea mays* var. “Pioneer 0725” FAO

500) was sown as a cash crop on June 10, 2021, and was irrigated using a sprinkler system throughout its cropping cycle, receiving a total of 700 mm of water (approximately 50 mm per week, split into two irrigation events each week). Maize was harvested on November 11, 2021. More detailed information about the experimental area and the agricultural practices developed during the experimental year can be found in García-Gutiérrez et al. (2024).

Microplot experimental design

The microplots established in the experimental area (1 m × 1 m) consisted of 20-cm-high stainless steel plates inserted into the soil to a depth of 10 cm. In mid-November 2020, nine microplots were established in the subplots, where maize residues from the previous cropping season were removed. Enriched ^{15}N maize residues (1.06% N, 42.6% C, 40.3 C:N ratio, 7.03% atom ^{15}N) obtained from a previous field experiment (Guardia et al. 2018) were manually incorporated into the microplot topsoil (0–15 cm depth) at a rate of 950 g m^{-2} , thereby delivering a total N rate of 100 kg N ha^{-1} . Afterward, barley and vetch cover crops were manually sown in the microplots at the same rate as that on the subplots (i.e., 20 g m^{-2}), and bare fallow was also applied to similar microplots. As with the surrounding subplots (and on the same dates), cover crops within each microplot were chemically terminated and then manually incorporated into the soil at a depth of 10–15 cm. Before maize fertilization, 18 additional microplots were established in the subplots, where the maize residues from the previous cropping season (i.e., non-enriched ^{15}N maize residues) were incorporated into the topsoil. Based on the grain yield from the previous cropping season (10 Mg ha^{-1}), combined with the N content of 0.44% found in maize stover (as indicated by data from a 2-year maize experiment under similar climatic and management conditions (Monistrol et al. 2024)) and a maize harvest index of 0.52 (Hütsch and Schubert 2017), the total gross N rate applied in the non-enriched ^{15}N maize residue microplots was estimated to be 41 kg N ha^{-1} . Six maize plants were included in each microplot. The 27 microplots were fertilized by applying a 2-L fertilizer solution per microplot at a rate of 210 kg N ha^{-1} on July 19, 2021. A homogeneous broadcast application was achieved using graduated cylinders and hand

sprayers, with the aid of 0.25 m^2 quadrants placed within each microplot, as described in García-Gutiérrez et al. (2023). The fertilizer solution for the nine initial microplots (i.e., those receiving ^{15}N -enriched maize residues) consisted of NH_4NO_3 (AN) at natural ^{15}N abundance. The 18 microplots in the subplots with non-enriched maize residues were fertilized with ^{15}N -enriched NH_4NO_3 (20% ^{15}N atom), with nine receiving $^{15}\text{NH}_4\text{NO}_3$ (^{15}AN) and the other nine receiving $\text{NH}_4^{15}\text{NO}_3$ (A^{15}N). Therefore, three replicates per treatment were established in a completely randomized block design. The experiment included three cover crop treatments (barley, B; vetch, V; and bare fallow, F) and three nitrogen sources (^{15}AN , A^{15}N , and ^{15}N -labeled maize residue (^{15}RES)). The experiment was conducted in two distinct periods. The first period (P1) began in November 2020 with the incorporation of ^{15}N -enriched maize residues into the soil. This period included the development of cover crops, which were chemically terminated and incorporated into the soil by mid-April. P1 ended before maize fertilization on July 19, 2021. The second period (P2) started with maize fertilization and ended with the maize harvest on November 17, 2021. An outline of the experimental design for P1 and P2 is shown in Figs. 1a and 1b, respectively.

Soil sampling and analysis

The topsoil (0–10 cm) was sampled using stainless steel cores (5 cm diameter). Before maize fertilization (P1), the soil was sampled monthly. The sampling frequency was increased up to four times per week during the 2 weeks after maize fertilization. Then, the sampling frequency was decreased to once per month until the end of the experiment. Fresh soil samples were stored at -20°C until further analysis.

The soil mineral N concentration (NH_4^+ and NO_3^-) was measured in all fresh soil samples after extraction with 1 M KCl (8:50, w/v) for 1 h at 180 rpm. This was followed by centrifugation at 4000 rpm for 10 min and final filtration using $0.45\text{-}\mu\text{m}$ cellulose syringe filters (Agilent Technologies, USA). The resulting soil extracts were frozen at -20°C until analysis. The mineral N was analyzed using automated colorimetric methods with a flow injection analyzer (FIA) coupled with a UV–vis spectrophotometer detector (FIAS 400, Perkin Elmer, and SFA FUTURA, AMS Alliance, for NH_4^+ and NO_3^- analyses, respectively).

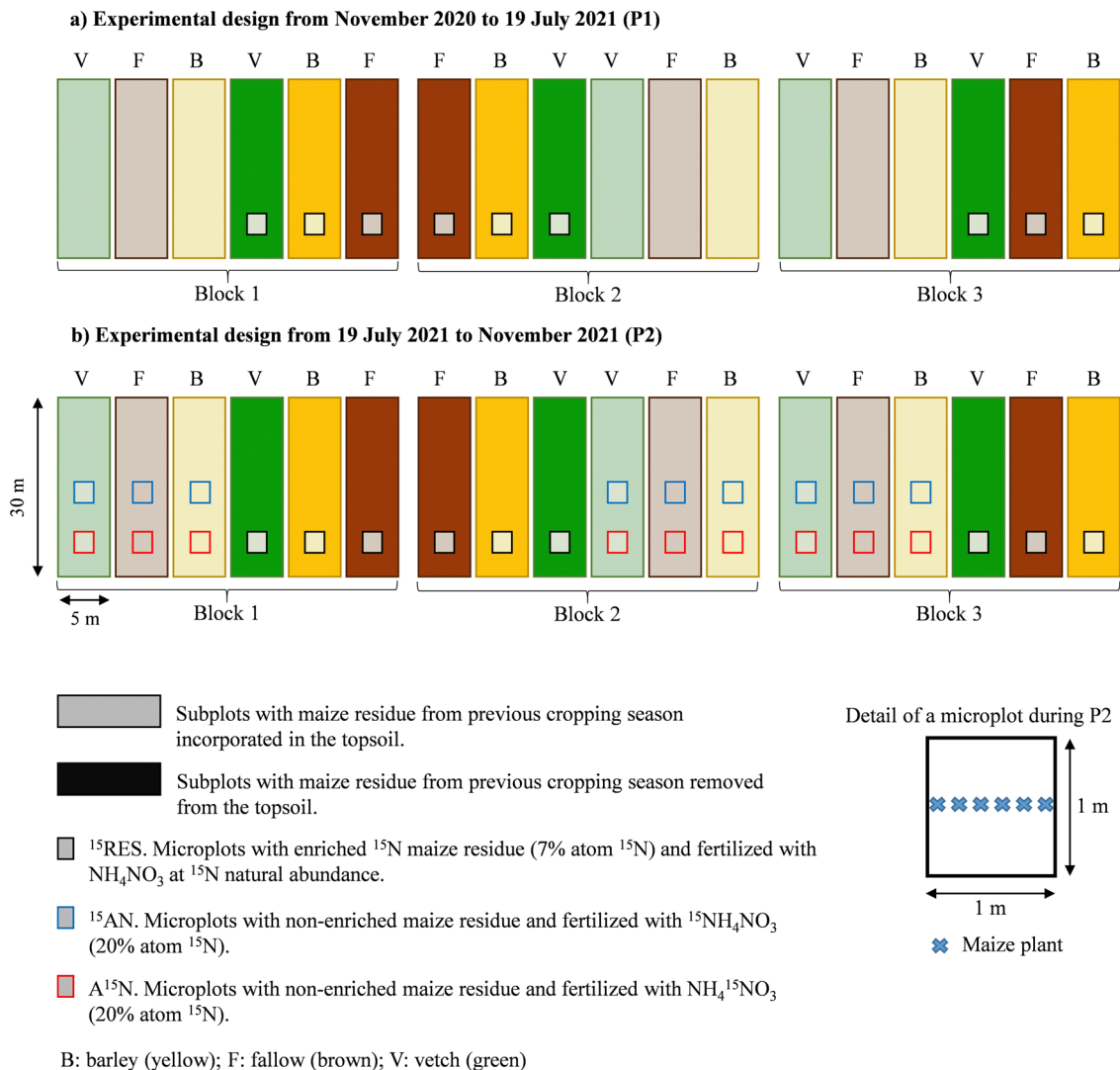


Fig. 1 Experimental design of the microplots field assay (**a**) before (P1) and (**b**) after maize fertilization (P2)

Reactions with the Griess reagent were used to analyze the NO_3^- -N concentration (Wang et al. 2016), and the NH_4^+ -N concentration in the extracts was analyzed using the indophenol method (Krom 1980).

DOC was extracted from fresh homogenized topsoil samples with ultrapure type I water (8:50, w/v) at 180 rpm for 1 h, centrifuged at 4000 rpm for 10 min, and filtered (Filterlab 1300/800). Soil extracts were frozen at -20°C until analysis. DOC was quantified using a total organic carbon analyzer (Analytik Jena multi N/C 3100) equipped with an infrared detector.

On November 5, the soil was sampled at three depths (0–20, 20–40, and 40–60 cm) using a stainless steel

auger. Fresh soil samples were homogenized, air dried, and ground in the laboratory using a ball mill (Retsch MM 400, Retsch GmbH, Haan, Germany). Total soil N and ^{15}N contents were analyzed using an isotope-ratio mass spectrometer (IRMS) at the Interdepartmental Research Service of the Universidad Autónoma de Madrid (SIDI-UAM).

The water-filled pore space (WFPS) was determined for all gas sampling dates as follows:

$$\text{WFPS}(\%) = \frac{H \times D_b}{1 - \frac{D_b}{D_p}} \times 100$$

where H is the gravimetric water content on a dry basis, D_b is the soil bulk density, and D_p is the soil particle density, which is assumed to be 2.65 g cm^{-3} . The gravimetric water content was determined by oven drying 5 g of fresh soil samples at 105°C for 24 h. The bulk density was determined by taking soil samples at different depths using 100-cm^3 stainless steel cores and oven drying them at 105°C for 24 h.

Greenhouse gas sampling, analysis and calculation

The soil–atmosphere exchange of N_2O and CO_2 was measured during the experiment using manual static chambers ($13 \text{ cm height} \times 35 \text{ cm diameter}$). Head-space gas samples were collected at 0, 30, and 60 min after chamber closure using a 100-mL plastic syringe and 20-mL gas chromatograph (GC) vials. The greenhouse gas (GHG) concentrations were analyzed using a GC analyzer (HP-6890 GC, HTC-3 Headspace autosampler, Agilent Technologies, Spain) with a μECD for analyzing N_2O and a FID for analyzing CO_2 (Recio et al. 2018). Daily GHG fluxes were calculated from the slope of the linear increase in gas concentration during the closure period. Cumulative GHG emissions during P1, P2, and the entire experimental period were calculated using trapezoidal integration with linear interpolation of daily fluxes (Venterea et al. 2020).

Gas samples during cover crop development and before maize fertilization (P1) were taken at least once every 2 weeks, with increased sampling frequency during rainfall events and after agricultural management practices (i.e., cover crop termination and incorporation into the soil). The aboveground biomass of the cover crops was placed inside the headspace of the chamber during sampling (Fig. S1a). During the first 2 weeks after maize fertilization, sampling frequency increased to as much as four times per week. After 1 month, the frequency decreased to at least once every 2 weeks. The chambers were positioned next to the maize rows during the maize growing phase (Fig. S1b).

$^{15}\text{N}_2\text{O}$ sampling, analysis and flux calculation

On the same dates when GHGs were sampled, pre-evacuated 12-mL exetainer GC vials (Labco Limited, Lampeter, UK) were filled with gas samples from the chamber headspace at $t=0$ min in one replicate of

each treatment and at $t=60$ min after closure in all chambers. The vials were then sent to the stable isotope facility of the University of California (Davis, CA, USA) to analyze the ^{15}N enrichment of N_2O . This analysis was conducted using a Thermo Scientific GasBench+PreCon trace gas concentration system interfaced with a Thermo Scientific Delta V Plus IRMS. Only the gas samples most relevant for understanding N_2O emission dynamics, based on N_2O concentrations measured by GC and key agronomic events, were selected for ^{15}N enrichment analysis (P1: 1 day after cover crop incorporation and 2, 8 and 34 days after maize sowing; P2: 1, 2, 4, 7, 9, 11, 15, 22, and 43 days after fertilization). This targeted sampling covered periods accounting for over 85% of the total cumulative N_2O emissions, ensuring a representative integration of emission patterns. The selected dates captured both high-flux events shortly after fertilization and later low-flux episodes enabling clear identification of contrasting N_2O source dynamics while minimizing potential bias from unmeasured low-emission periods.

Equations 1–13 in Table S1 (adapted from Li et al. (2016) and Xu et al. (2019)) were used to calculate daily N_2O –N fluxes ($\text{mg N m}^{-2} \text{ d}^{-1}$) from the different sources studied: ^{15}AN fertilizer ($\text{N}_2\text{O}-\text{N}_{15\text{AN}}$), A^{15}N fertilizer ($\text{N}_2\text{O}-\text{N}_{\text{A}15\text{N}}$), ^{15}N -enriched maize residues ($\text{N}_2\text{O}-\text{N}_{15\text{RES}}$), and endogenous soil N ($\text{N}_2\text{O}-\text{N}_{\text{soil}}$). These equations also determined the proportion of N_2O emissions derived from each source, the N_2O EF, and other parameters related to $^{15}\text{N}_2\text{O}$ analysis. Calculations assumed that N_2O in the chamber headspace was a mixture of atmospheric N_2O present at chamber closure and N_2O emitted from the soil.

Plant sampling and analysis

Aboveground biomass samples of barley and vetch cover crops grown in the initial microplots with ^{15}N -enriched maize residues (^{15}RES) (Fig. 1a) were harvested on April 14, 2021, using a 0.096-m^2 frame. The remaining biomass in the microplots was left for chemical termination. Furthermore, representative root samples from the cover crops in the ^{15}RES microplots were collected. The soil was separated from the roots in the laboratory. The roots were then rinsed with tap water for 5 min and washed in an ultrasound-assisted bath for 10 min with deionized water (García-Gómez et al. 2015). All plant material

was oven dried at 70 °C for 48 h and ground using a Retsch MM 400 ball mill (Retsch GmbH, Haan, Germany). The aboveground biomass yield was calculated based on the 0.096-m² frame area used for harvesting, whereas the root biomass yield was estimated according to the method described by Hu et al. (2018). The total C content in the aboveground biomass of the cover crops was determined using the Dumas method and an elemental analyzer (TruMac CN Leco®, USA).

Maize was harvested at physiological maturity on November 19, 2021. The aboveground biomass of the six plants in each microplot was measured by fresh weight in the field. To estimate the crop yield on a dry basis, the stover and grain moisture were determined by oven drying subsamples at 70 °C until a constant weight was obtained. The aboveground biomass (stover and grain) of the four central plants and the root of one of the central plants in each microplot were used to analyze the total N and ¹⁵N contents. After separating the soil from the roots, the roots were cleaned using the same procedure described above for the cover crop roots and oven dried at 70 °C for 48 h. The total N and ¹⁵N contents in the cover crops (aboveground and belowground biomass) and maize (roots, grain, and stover) samples were analyzed by IRMS at SIdI-UAM.

Calculation of N recovery in cover crops, maize, and soil

The plant recovery of N from the ¹⁵AN fertilizer ($plant_NR_{15AN}$), A¹⁵N fertilizer ($plant_NR_{A15N}$), combined fertilizer ($plant_NR_{15AN} + plant_NR_{A15N}$), and ¹⁵N-enriched maize residues ($plant_NR_{15RES}$) in maize at harvest was calculated using Eqs. 14–24 in Table S2 (adapted from Taveira et al. (2020)). Equations 22–24 in Table S2 were also used to calculate the recovery from ¹⁵N-enriched maize residues in cover crop biomass. The total N uptake in the different cover crops or maize plant tissues ($plant_TN$, kg N ha⁻¹) was calculated by multiplying the total N content in each cover crop or maize tissue (%) by the yield on a dry basis (kg plant tissue ha⁻¹) and dividing the result by 100.

The N recovery from the ¹⁵AN fertilizer in the soil ($soil_NR_{15AN}$), A¹⁵N fertilizer ($soil_NR_{A15N}$), and combined fertilizer ($soil_NR_{15AN} + plant_NR_{A15N}$) was calculated using Eqs. 25–32 in Table S3. The amount

of N in the different soil layers ($soil_TN$, kg N ha⁻¹) was calculated by multiplying the N content (%) in each soil layer by its bulk density measured at the end of the experiment (1.46 ± 0.07 , 1.52 ± 0.03 , and 1.55 ± 0.01 g cm⁻³ for the 0–20, 20–40 and 40–60 cm depths, respectively) and by the soil thickness of each layer. Then, the total N recovery from the fertilizer was calculated by adding $plant_NR_{fert}$ and $soil_NR_{fert}$.

Statistical analysis

Data analysis was performed using Statgraphics® Centurion 19 and R Studio 4.3.2 software. Data distribution normality and variance uniformity were verified in the studied variables using the Shapiro–Wilk and Levene’s tests, respectively. When necessary, inverse or log transformations were applied before the analysis. Analyses of variance were conducted using generalized linear models (considering the randomized block design) to assess significant differences between cover crops in the studied variables, except for meteorological variables. The least significant difference (LSD) test at $P < 0.05$ was used for multiple comparisons between means. Pearson’s correlation analysis was performed to assess the relationships between daily N₂O, ¹⁵N₂O, and CO₂ emissions, soil mineral N and DOC concentrations, WFPS, and soil and air temperatures for the two experimental periods and for the entire experimental period.

Results

Meteorological conditions, soil mineral N, and DOC

The cumulative rainfall during the experiment (from mid-November 2020 to mid-November 2021) was 417 mm, with 230 and 187 mm occurring during P1 and P2, respectively. The average temperature during the experimental year was 14.3 °C, with minimum and maximum air temperatures of –16.3 °C and 41.6 °C reported in mid-January and mid-August, respectively (Fig. S2). The average WFPS ranged from 13 to 52% before maize irrigation and from 15 to 64% during the maize irrigation period (Fig. 2a).

The average soil NH₄⁺ concentrations increased after maize fertilization, rising from 4 to 67 mg N kg⁻¹, and then decreased to baseline levels 3 weeks after fertilization (Fig. 3a). Cover

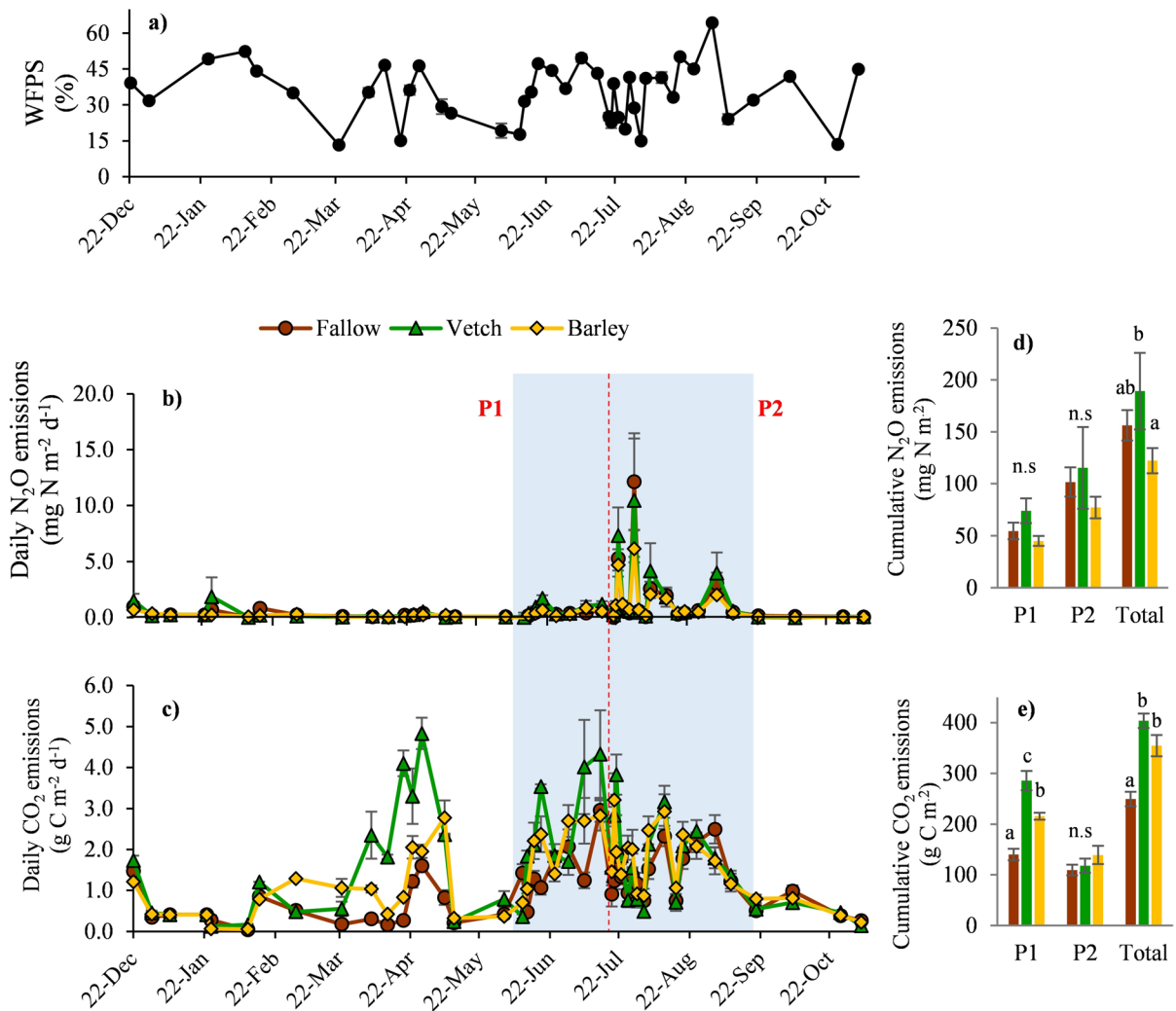


Fig. 2 Daily topsoil (10 cm depth) water-filled pore space (WFPS) (a), N₂O emissions (b) and CO₂ emissions (c) before (P1) and after maize fertilization (P2) in the different cover crop treatments (bare fallow, vetch, and barley). The dotted red line indicates the maize top-dressing fertilization date (July 19, 2021), corresponding to the transition from P1 to P2. The blue shadow area corresponds to the maize irrigation period. Cumu-

lative N₂O (d) and CO₂ (e) emissions during P1 and P2 and total cumulative emissions at the end of the experiment. Different letters denote statistical differences ($P < 0.05$) between cover crop treatments within each specific period (P1, P2, or total) by applying the LSD test. n.s.: no significant. Vertical bars denote the standard error of the mean ($n = 3$ in P1, $n = 9$ in P2)

cropping treatments did not influence soil NH_4^+ concentrations throughout the entire experimental period (Fig. S3a). Soil NO_3^- concentrations also increased within 4–9 days after fertilization, reaching 122, 146, and 110 mg N kg⁻¹ in the F, V, and B treatments, respectively (Fig. 3b). Before maize fertilization (P1), B microplots showed significantly lower soil NO_3^- concentrations than V or F

(Fig. S3b). The average soil NO_3^- concentration in V during P2 (82.0 mg N kg⁻¹) was 36% and 33% higher than that in F and B, respectively ($P < 0.05$). No significant differences in soil DOC concentrations were observed between the treatments (Fig. S3c), with average values ranging from 20.1 to 51.4 mg C kg⁻¹ soil over the entire experimental period (Fig. 3c).

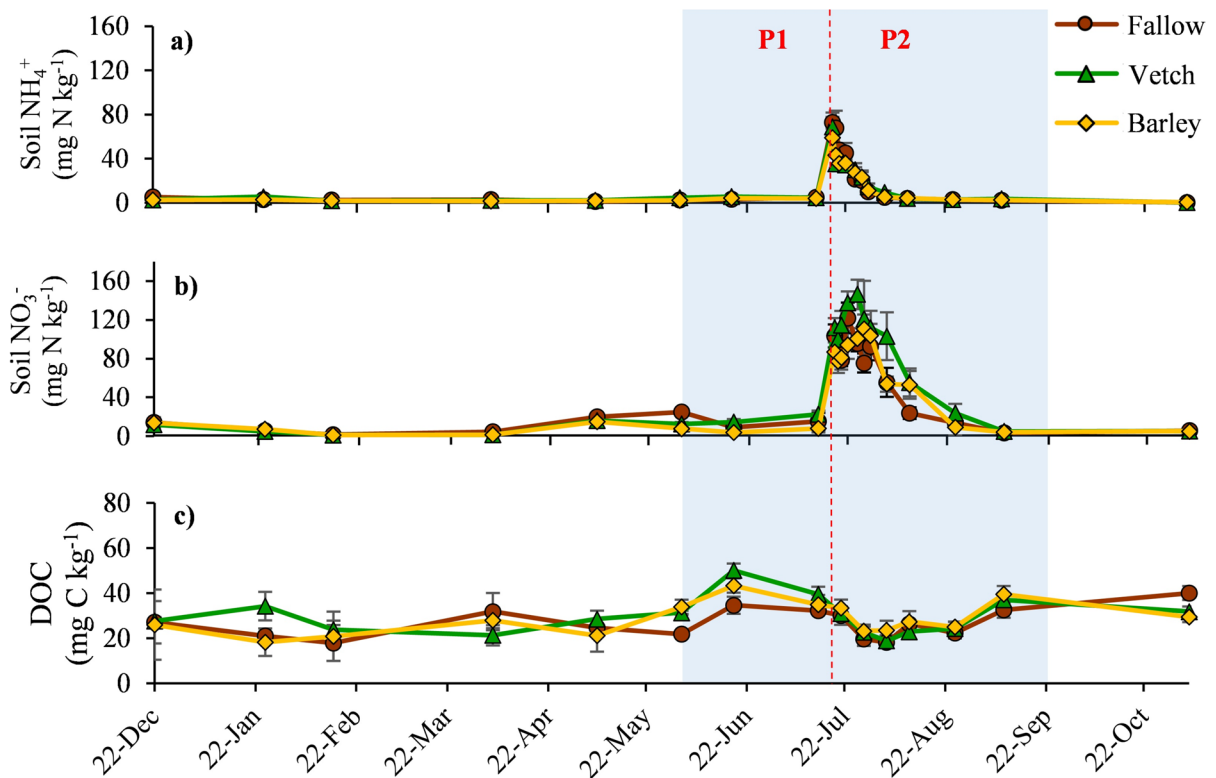


Fig. 3 Daily soil NH_4^+ (a), NO_3^- (b), and DOC (c) concentrations before (P1) and after maize fertilization (P2) in the different cover crop treatments (bare fallow, vetch and barley). Vertical bars denote the standard error of the mean ($n=3$ in

P1, $n=9$ in P2). The dotted red line indicates the maize top-dressing fertilization date (July 19, 2021), corresponding to the transition from P1 to P2. The blue shadow area corresponds to the maize irrigation period

N_2O and CO_2 emissions

Before maize fertilization (P1), most daily N_2O fluxes were below $0.5 \text{ mg N m}^{-2} \text{ d}^{-1}$ (Fig. 2b). The average accumulated N_2O emission was 57.9 mg N m^{-2} , with no significant differences between cover cropping treatments (Fig. 2d). Daily N_2O emissions were positively correlated with WFPS ($r=0.53$, $P<0.01$), CO_2 emissions ($r=0.53$, $P<0.05$), and soil NH_4^+ ($r=0.77$, $P<0.05$) (Fig. S4a).

Main N_2O increments during P2 (Fig. 2b) were reported after fertilization (reaching $9.6 \text{ mg N m}^{-2} \text{ d}^{-1}$), and after a heavy rainfall event at September 2 (reaching $3.0 \text{ mg N m}^{-2} \text{ d}^{-1}$). The average accumulated N_2O emission flux during P2 was 98.0 mg N m^{-2} , with no significant differences between cover cropping treatments (Fig. 2d). At the end of both P1 and P2, V significantly increased the cumulative N_2O emissions (i.e., by 47%, Fig. 2d) with respect

to barley ($P<0.05$). During P2, daily N_2O emissions were strongly and positively correlated with both air and soil temperatures and CO_2 emissions ($r=0.66$, $P<0.01$; $r=0.66$, $P<0.01$; and $r=0.80$, $P<0.001$, respectively) (Fig. S4b). These correlations were also significant considering the entire experimental period, with additional correlations ($P<0.05$) found between N_2O emissions and soil WFPS and NH_4^+ ($r=0.31$ and $r=0.48$, respectively) (Fig. S4c).

During P1, cumulative CO_2 emissions were 104% and 33% higher in V than in F and B, respectively (Fig. 2e). Considering the entire experimental period, cumulative CO_2 emissions were lower in F (249 g C m^{-2}) than in V and B (404 and 355 g C m^{-2} , respectively; $P<0.05$) (Fig. 2e). Similar to the daily N_2O fluxes, air and soil temperatures were positively correlated with daily CO_2 emissions throughout the whole experiment (Fig. S4c), particularly during P2

($r=0.72$, $P<0.001$, and $r=0.71$, $P<0.001$, respectively) (Fig. S4b).

$^{15}\text{N}_2\text{O}$ emissions

Before maize fertilization (P1), the contribution of maize residues on N_2O emissions increased from 2% of the daily N_2O fluxes at the beginning of the experiment to 7% after maize sowing on June 11, 2021 (Fig. 4). During this period, the cover cropping treatments did not have a significant effect on emission from ^{15}N -enriched maize residues ($\text{N}_2\text{O}-\text{N}_{15\text{RES}}$) or soil endogenous N ($\text{N}_2\text{O}-\text{N}_{\text{soil}}$) (Table S4). Nitrous oxide emissions derived from soil were strongly and positively correlated with WFPS ($r=0.99$, $P<0.001$, $n=5$), and $\text{N}_2\text{O}-\text{N}_{15\text{RES}}$ emissions were correlated with DOC ($r=0.99$, $P<0.01$, $n=5$) during P1 (Fig. S4a).

After fertilization (P2), the N_2O emissions derived from the different sources (i.e., maize residues, fertilizer, and soil N) were not affected by the incorporation of the different cover crops (Table S5). $^{15}\text{N}_2\text{O}$

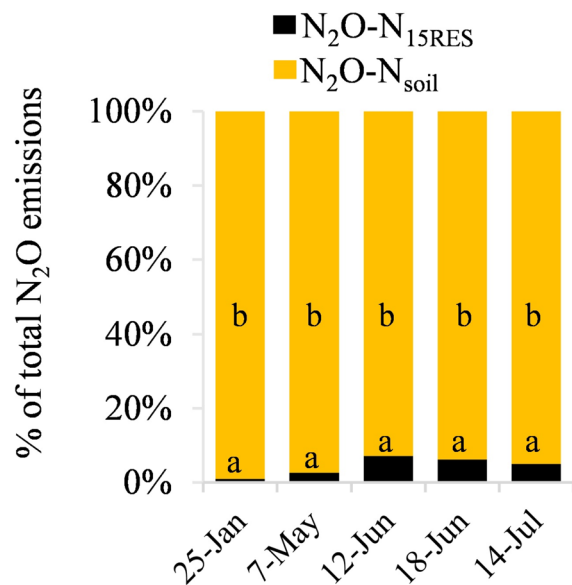


Fig. 4 Proportion of daily N_2O emissions derived from ^{15}N -enriched maize residues ($\text{N}_2\text{O}-\text{N}_{15\text{RES}}$) and soil endogenous N ($\text{N}_2\text{O}-\text{N}_{\text{soil}}$) in gas samples collected during P1 (from maize residue incorporation on November 17, 2020, and until maize fertilization on July 19, 2021). Different lowercase letters denote significant difference between N_2O sources in the different sampling dates by applying the LSD test at the 95% confidence level ($n=9$)

analyses also showed that the ^{15}AN and A^{15}N fertilizers contributed 42%–61% and 9%–18%, respectively, to daily N_2O emissions during the first 2 weeks after fertilization. Main emission peaks (Fig. 2b) coincided with elevated contributions from ^{15}AN . Regarding maize residues, $\text{N}_2\text{O}-\text{N}_{15\text{RES}}$ increased from 1 to 3% of total N_2O emissions at 16 days after fertilization (Fig. 5). The contribution of endogenous soil N to N_2O emissions during the first 2 weeks after fertilization was, on average, 28% of daily N_2O emissions, increasing thereafter to reach a 78% contribution in September 2. The average N_2O EFs from fertilizer and maize residues were 0.23% and 0.05%, respectively.

Nitrogen recovery in the plant

Nitrogen recovery from maize residues in cover crops

The N content and total plant N in the aboveground biomass of cover crops were 2.4 and 2.1 times higher in V than in B ($P<0.05$). Vetch also tripled the N concentration and N yield in belowground biomass compared with B. The average N recovered from maize residues in cover crop biomass accounted for 1.1% (Table 1). No differences were reported in the N uptake from ^{15}RES despite the ^{15}N enrichment being higher in B than in V ($P<0.05$, Table 1).

Nitrogen recovery in maize

The average N uptake of maize at harvest was 76, 153, and 16 kg N ha^{-1} in stover, grain, and roots, respectively, with no significant differences between cover crop treatments, consistent with findings for yield and plant N concentrations (Table 2). The average ^{15}N content in all maize tissues under the ^{15}RES treatment was lower than in treatments receiving ^{15}N -enriched fertilizer, with higher values observed in A^{15}N than in ^{15}AN (i.e., by 69%, 68%, and 61% higher in stover, grain, and roots, respectively) (Table S6). A 7.7% N recovery from ^{15}N -enriched maize residues was observed in the subsequent maize crop, without significant differences between cover crop treatments (Fig. S5).

The average N uptake from fertilizer (plant N_{fert}) at maize harvest was 19.3, 43.1, and 4.7 kg N ha^{-1} in stover, grain, and roots, respectively (Table 3). The plant N uptake derived from the A^{15}N fertilizer

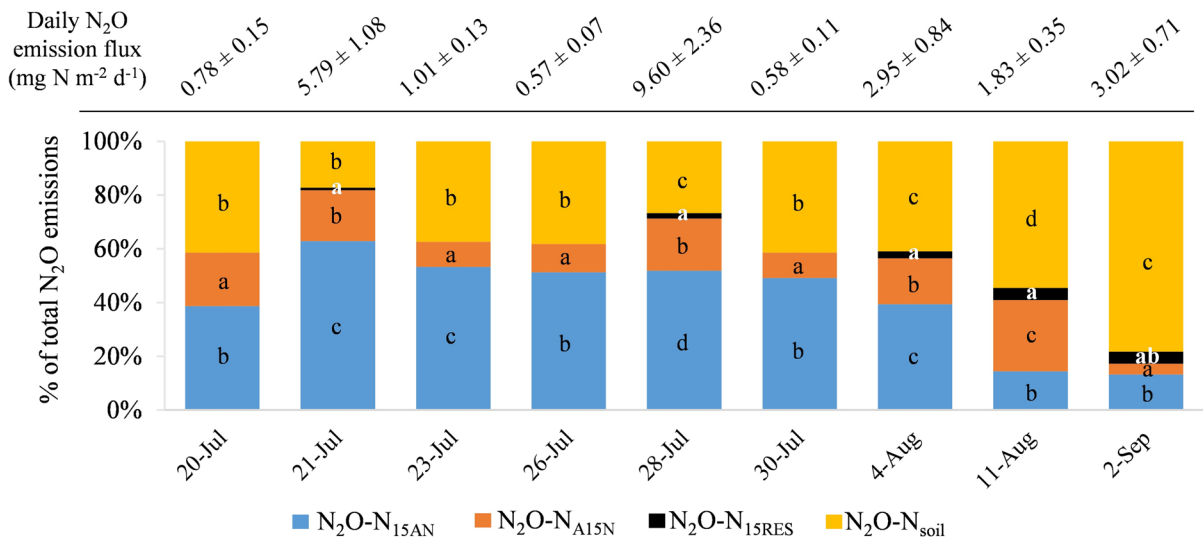


Fig. 5 Proportion of daily N₂O emissions derived from the ¹⁵NH₄NO₃ fertilizer (N₂O-N_{15AN}), the NH₄¹⁵NO₃ fertilizer (N₂O-N_{15N}), ¹⁵N-enriched maize residues (N₂O-N_{15RES}), and soil endogenous N (N₂O-N_{soil}) during P2 (i.e., after maize fertilization on July 19, 2021). Different letters denote significant differences between N₂O sources in the different sampling dates by applying the LSD test at the 95% confidence level (*n*=9). Data above bars indicate the average daily N₂O emission flux on each sampling date (*n*=27)

Table 1 Aboveground and belowground cover crop production (Mg DW ha⁻¹), C and N contents (%), total N (kg N ha⁻¹), atom ¹⁵N %, and N derived from ¹⁵N-enriched maize residues

Aboveground biomass								
Cover crop	Production (Mg DW ha ⁻¹)	N content (%)	Total N (kg N ha ⁻¹)	¹⁵ N (%)	N _{15RES} (kg N ha ⁻¹)	N _{soil} (kg N ha ⁻¹)	C content (%)	N recovery (%)
Vetch	2.9 ± 0.4	3.69 ± 0.04 b	106 ± 14	0.42 ± 0.01 a	0.79 ± 0.04	105 ± 14	41.3 ± 0.5	0.79 ± 0.04
Barley	3.4 ± 0.6	1.52 ± 0.05 a	51 ± 7	0.49 ± 0.02 b	0.89 ± 0.11	50 ± 7	41.1 ± 0.3	0.88 ± 0.11
<i>P</i> value	0.530	0.001	0.083	0.045	0.393	0.082	0.460	0.401
Belowground biomass								
Cover crop	Production (Mg DW ha ⁻¹)	N content (%)	Total N (kg N ha ⁻¹)	¹⁵ N (%)	N _{15RES} (kg N ha ⁻¹)	N _{soil} (kg N ha ⁻¹)	N recovery (%)	
Vetch	1.1 ± 0.0	2.03 ± 0.03 b	21.4 ± 0.3 b	0.44 ± 0.00 a	0.25 ± 0.01	21.1 ± 0.3 b	0.25 ± 0.01	
Barley	1.1 ± 0.0	0.64 ± 0.06 a	6.8 ± 0.7 a	0.65 ± 0.03 b	0.28 ± 0.02	6.5 ± 0.3 a	0.28 ± 0.02	
<i>P</i> value	¹	0.002	0.001	0.024	0.430	0.001	0.422	

Values are expressed as the mean ± standard error (*n*=3). Different letters denote significant differences between cover crops by applying the LSD test at the 95% confidence level

¹ Belowground biomass production was obtained from Hu et al. (2018)

(46.4 kg N ha⁻¹) was 2.3 times higher than that derived from the ¹⁵AN fertilizer (average 20.6 kg N ha⁻¹) (Table 3). Barley cover crop increased

plant_N_{15AN} in grain (by 39% and 33% compared with F and V, respectively, *P*<0.05) and in roots (by 31% and 55% compared with F and V, respectively,

Table 2 Maize yield (Mg ha⁻¹), N content (%), and N uptake (kg N ha⁻¹) in different plant tissues (stover, grain, and roots) in different cover crop treatments (F, bare fallow; V, vetch; B, barley)

Cover crop treatment	Maize yield (Mg ha ⁻¹)			Maize N content (%)			Maize N uptake (kg N ha ⁻¹)		
	Stover	Grain	Root	Stover	Grain	Root	Stover	Grain	Root
F	10.1 ± 0.3	10.5 ± 0.3	1.96 ± 0.05	0.72 ± 0.02	1.41 ± 0.02	0.75 ± 0.03	72.9 ± 3.1	147 ± 3	14.7 ± 0.6
V	10.0 ± 0.5	10.3 ± 0.6	1.93 ± 0.06	0.71 ± 0.04	1.42 ± 0.02	0.78 ± 0.03	71.5 ± 5.9	145 ± 3	15.0 ± 2.0
B	11.7 ± 0.9	11.5 ± 1.6	2.22 ± 0.16	0.70 ± 0.02	1.43 ± 0.02	0.81 ± 0.03	83.4 ± 6.8	165 ± 10	17.9 ± 1.4
<i>P</i> value	0.361	0.248	0.922	0.807	0.742	0.146	0.425	0.202	0.100

Values are expressed as the mean ± standard error (*n*=9). Different letters denote significant differences between treatments by applying the LSD test at the 95% confidence level

0.05 < *P* < 0.10). Furthermore, B significantly increased stover_N_{A15N} and root_N_{A15N} compared with F (by 94% and 76%, respectively) and V (by 70% and 34%, respectively) (Table 3). Consequently, the percentage of plant N recovery from the fertilizer was higher in B (42%) than in F (26%), with V showing intermediate results (30%) (Fig. 6).

Nitrogen recovery in the soil

No differences were found between the soil N derived from ¹⁵AN (average 20.5 kg N ha⁻¹) and A¹⁵N fertilizers (average 25.0 kg N ha⁻¹) (Table 4). Considering the whole 0–60 cm depth, B significantly increased soil_N_{15AN} compared with V (by 84%) and F (by 123%). However, no differences were observed between treatments in the sum of both soil_N_{15AN} and soil_N_{A15N} (i.e., N_{fert}) (Table 4), with an average soil N recovery of 22% (Fig. 6). Barley was the only treatment that resulted in significant differences between plant_NR_{fert} (42%) and soil_NR_{fert} (23%). Total fertilizer N recovery, calculated as the sum of plant- and soil-derived ¹⁵N, was highest in the B treatment, followed by the V treatment, and lowest in the F treatment (Fig. 6).

Discussion

Effects of cover crops on N₂O and CO₂ emissions

When the total cumulative emissions are considered, plots with vetch as a cover crop resulted in higher N₂O emissions compared with barley (Fig. 2d). These results partially support those from the meta-analysis

by Basche et al. (2014), who observed an overall neutral effect of cover crops on annual N₂O emissions, although the N₂O increment was significant for legume species and during the cover crop decomposition and cash crop phases, particularly when residues were incorporated.

During cover cropping period, it has been argued that the active rhizodeposition (Li et al. 2023b; Guardia et al. 2025) and decomposition of N-rich vetch residues may increase SOC and N inputs and NH₄⁺ concentration, thereby favoring nitrification/denitrification processes (Daly et al. 2024). This was indeed supported by the positive correlation between daily NH₄⁺ and N₂O emissions (Fig. S4c) and by the higher soil NO₃⁻ concentration in vetch plots compared with fallow and barley after subsequent maize fertilization (Fig. S3b). Low N₂O fluxes during the growing period, limited by soil moisture and temperatures (García-Marco et al., 2014; Sanz-Cobena et al. 2014; Guardia et al. 2016), may contribute to the lack of significant differences during this period, even though our results may suggest a legacy effect of cover crop species during not only the post-incorporation phase but also during the subsequent cash crop period, thus highlighting the relevance of considering the whole annual rotation.

In accordance with the meta-analysis by Muhammad et al. (2019), CO₂ emissions increased in the presence of cover crops when considering the entire experiment, compared with bare fallow (Fig. 2e), thus showing an increased root biomass, respiration, and microbial activity. Our findings were supported by eg., Chirinda et al. (2010) or Sanz-Cobena et al. (2014), who reported higher CO₂ emissions in legume cover crops than in nonlegume cover crops,

Table 3 Nitrogen derived from ^{15}N -enriched maize residues ($\text{N}_{15\text{RES}}$), $^{15}\text{NH}_4\text{NO}_3$ fertilizer ($\text{N}_{15\text{AN}}$), $\text{NH}_4^{15}\text{NO}_3$ fertilizer (N_{A15N}), and endogenous soil N (N_{soil}) in the different maize tissues (stover, grain, and roots) and in total maize plants in different cover crop treatments (bare fallow, vetch, and barley). Data are expressed in kg N ha^{-1}

Cover crop treatment				
Stover	Fallow	Vetch	Barley	
$\text{N}_{15\text{RES}}$	2.55 ± 0.12 A	2.13 ± 0.08 A	2.91 ± 0.32 A	0.157
$\text{N}_{15\text{AN}}$	4.85 ± 0.01 B	5.75 ± 1.50 B	6.04 ± 0.59 B	0.725
N_{A15N}	10.1 ± 1.5 C a	11.6 ± 1.1 C a	19.6 ± 1.7 C b	0.022
N_{soil}	55.4 ± 1.6 D	52.1 ± 3.6 D	54.9 ± 4.5 D	0.826
	0.000	0.000	0.000	<i>P</i> value
Grain	Fallow	Vetch	Barley	
$\text{N}_{15\text{RES}}$	4.40 ± 0.21 A	4.73 ± 0.21 A	4.35 ± 0.03 A	0.202
$\text{N}_{15\text{AN}}$	11.8 ± 0.2 B a	12.3 ± 1.2 B a	16.4 ± 1.2 B b	0.014
N_{A15N}	23.5 ± 3.1 C	28.1 ± 0.9 C	37.2 ± 3.6 C	0.104
N_{soil}	108 ± 3 D	100 ± 2 D	107 ± 6 D	0.423
	0.000	0.000	0.000	<i>P</i> value
Root	Fallow	Vetch	Barley	
$\text{N}_{15\text{RES}}$	0.65 ± 0.01 A	0.69 ± 0.09 A	0.71 ± 0.18 A	0.909
$\text{N}_{15\text{AN}}$	1.50 ± 0.03 B ab	1.27 ± 0.18 B a	1.97 ± 0.11 B b	0.066
N_{A15N}	2.29 ± 0.21 C a	3.02 ± 0.52 C a	4.03 ± 0.25 C b	0.015
N_{soil}	10.3 ± 0.7 D	10.1 ± 1.3 D	11.2 ± 1.6 D	0.474
	0.000	0.000	0.000	<i>P</i> value
Plant	Fallow	Vetch	Barley	
$\text{N}_{15\text{RES}}$	7.59 ± 0.32 A	7.55 ± 0.36 A	7.96 ± 0.48 A	0.782
$\text{N}_{15\text{AN}}$	18.1 ± 0.2 B	19.3 ± 2.3 B	24.4 ± 1.7 B	0.105
N_{A15N}	35.9 ± 4.8 C a	42.7 ± 2.1 C ab	60.8 ± 5.1 C b	0.054
N_{soil}	173 ± 4 D	162 ± 7 D	173 ± 8 D	0.561
	0.000	0.000	0.000	<i>P</i> value

Values are expressed as the mean $\pm$ standard error ($n=3$). Different capital letters denote significant differences between N sources, and different lowercase letters denote significant differences between cover crop treatments by applying the LSD test at the 95% confidence level

which could be related to an increase in soil microbial community abundance and rhizosphere respiration (Muhammad et al. 2021).

Sources of N_2O emissions

In this study, we used distinct N sources of contrasting availability throughout the cropping period:

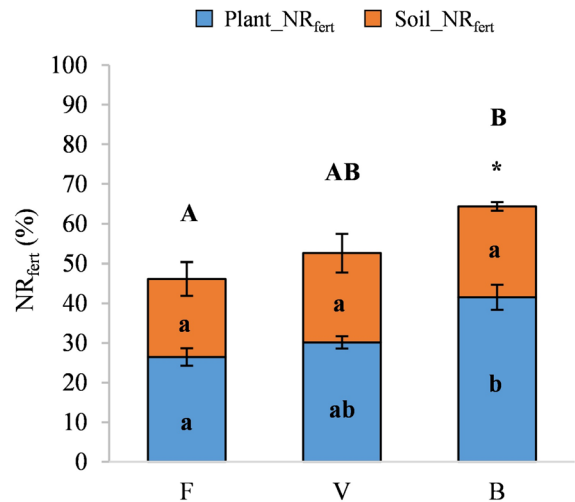


Fig. 6 Nitrogen recovery from the NH_4NO_3 fertilizer in maize plants at harvest ($\text{Plant_NR}_{\text{fert}}$, %) and in soil at the end of the experiment ($\text{Soil_NR}_{\text{fert}}$, %) in the different cover crop treatments (F, bare fallow; V, vetch; B, barley). Different lowercase letters denote significant differences between cover crop treatments in plant or soil NR_{fert} . Different capital letters denote significant differences between cover crop treatments in total NR_{fert} ($\text{plant_NR}_{\text{fert}} + \text{soil_NR}_{\text{fert}}$). *Significant differences in the N recovery between plant ($\text{Plant_NR}_{\text{fert}}$) and soil ($\text{Soil_NR}_{\text{fert}}$) in each cover crop treatment (F, V, or B). Statistical differences were obtained by applying the LSD test at the 95% confidence level. Vertical bars denote the standard error of the mean ($n=9$)

crop residues (maize stover with a high C:N ratio, incorporated at the beginning of P1, and vetch and barley as cover crops, incorporated as green manure at the end of P1), and mineral N supplied by a synthetic fertilizer at the beginning of P2. The chemical characteristics of recalcitrant crop residues, such as high C:N ratios and high cellulose, hemicellulose, and lignin fractions (Abalos et al. 2022b), are related to their slow biodegradability rates (Jensen et al. 2005; Lashermes et al. 2022). Therefore, we could successfully confirm our initial hypothesis that maize residues would have a low contribution to N_2O emissions. The increasing relative contribution of maize stover to N_2O fluxes after maize irrigation (Fig. 4) and during P2 indicated enhanced mineralization rates of the previous maize stover under higher temperature and soil moisture conditions, as supported by the positive correlations between N_2O – $\text{N}_{15\text{RES}}$ fluxes and WFPS (Figs. S4b and S4c). Consistent with our findings, Ferrari Machado et al. (2021) also reported a low

Table 4 Soil N derived from $^{15}\text{NH}_4\text{NO}_3$ fertilizer ($\text{soil_N}_{15\text{AN}}$) and $\text{NH}_4^{15}\text{NO}_3$ fertilizer ($\text{soil_N}_{\text{A15N}}$) at three soil sampling depths (0–20, 20–40, and 40–60 cm), considering the entiresoil sampling depth (0–60 cm), and total soil N derived from fertilizer ($\text{soil_N}_{\text{fert}}$) at a depth of 0–60 cm in the different cover crop treatments (F, bare fallow; V, vetch; B barley)

Cover crop treatment	0–20 cm		20–40 cm		40–60 cm		Total (0–60 cm)		
	$\text{Soil_N}_{15\text{AN}}$ (kg N ha $^{-1}$)	$\text{Soil_N}_{\text{A15N}}$	$\text{Soil_N}_{15\text{AN}}$ (kg N ha $^{-1}$)	$\text{Soil_N}_{\text{A15N}}$	$\text{Soil_N}_{15\text{AN}}$ (kg N ha $^{-1}$)	$\text{Soil_N}_{\text{A15N}}$	$\text{Soil_N}_{15\text{AN}}$ (kg N ha $^{-1}$)	$\text{Soil_N}_{\text{A15N}}$	N_{fert}
F	4.20 ± 0.74	6.19 ± 0.74	4.71 ± 2.21	11.8 ± 6.7	4.94 ± 2.68 ab	9.52 ± 3.65	13.8 ± 4.5 a	27.5 ± 10.2	41.3 ± 8.9
V	10.5 ± 3.9	5.50 ± 1.92	3.45 ± 0.88	9.70 ± 3.66	2.71 ± 0.63 a	15.3 ± 6.0	16.7 ± 5.3 a	30.5 ± 10.1	47.2 ± 10.2
B	9.63 ± 2.59	6.04 ± 0.72	10.3 ± 3.3	4.29 ± 1.62	10.9 ± 3.1 b	6.83 ± 0.23	30.8 ± 3.7 b	17.2 ± 1.4	48.0 ± 2.3
<i>P</i> value	0.287	0.878	0.068	0.621	0.045	0.501	0.009	0.752	0.842

Values are expressed as the mean ± standard error ($n=3$). Different letters denote significant differences between cover crop treatments by applying the LSD test at the 95% confidence level

contribution of crop residues (ranging from 0.3% to 1.3%) to total N_2O emissions in a 4-year crop rotation.

Considering the maize fertilization period and the information from $^{15}\text{AN}/\text{A}^{15}\text{N}$ microplots, our results align with those previously reported by García-Gutiérrez et al. (2023) and Guardia et al. (2018), where NH_4^+ was identified as the primary source of N_2O emissions in ^{15}N tracing experiments when NH_4NO_3 was applied to Mediterranean agricultural soils. This supports the third hypothesis of this study, suggesting that NH_4^+ –N oxidation-based processes (nitrification, nitrifier denitrification, or nitrification coupled with denitrification) could be relevant pathways leading to N_2O emissions from the exogenous N applied to maize. Our findings in line with the global meta-analysis conducted by Han et al. (2024), who reported higher N_2O emissions from nitrification than from denitrification in cropland experiments using ^{15}N -labeled fertilizers. Besides, He et al. (2025) reported a significant contribution of nitrifier denitrification to N_2O emissions in wet soils, particularly at intermediate WFPS values (60%–70%), which could be reached during the repeated rewetting episodes in the maize irrigation period. However, further research is needed regarding the contribution of this process in semiarid soils, using an enriched dual isotope (^{15}N – ^{18}O) approach as a suitable technique (Wrage-Mönnig et al. 2018). The continuous changes in soil pore aerobicity and mobility of NO_3^- ions—from aerobic environments (where is produced through

nitrification) to anaerobic microsites—also support the suitability of the coupling of nitrification and denitrification processes.

In addition to fertilizer, our results suggest that the contribution of endogenous soil N to N_2O emissions (including the quantitatively relevant N mineralized from cover crop residues and N released from soil due to the priming effect following synthetic N addition or biological N fixation) should not be neglected. In this study, endogenous soil N accounted for approximately 40% of N_2O emissions even immediately after N fertilizer application (Fig. 5). The application of organic amendments, such as cover crop residues, can modify the magnitude of N_2O priming by introducing labile C sources for denitrifiers, increasing the availability of mineral N substrates, modifying microbial communities (Arcand and Congreves 2020; Daly et al. 2024), and increasing soil moisture (Roman-Perez and Hernandez-Ramirez 2021). This effect could contribute to explain the higher emissions in vetch than in barley (+47%, Fig. 2d) through the priming effect of N-rich vetch residues and labile-C rhizodeposits stimulating soil mineralization (Holz et al. 2023). Other studies that applied ^{15}N -labeled fertilizers in calcareous soils under Mediterranean climates, such as those by Guardia et al. (2018) in irrigated maize and García-Gutiérrez et al. (2023) in rainfed barley, have also reported a significant contribution of endogenous N in soil to N_2O emissions, even though cover crop residues were not included in these studies.

Nitrogen recovery from maize residues and fertilizer

The slow degradation and mineralization of recalcitrant maize residues resulted in the incorporation of most of the N in the cover crop biomass derived from sources other than the residues themselves, such as endogenous soil N or biological N fixation (Table 1). The increase in plant ^{15}N concentrations (derived from ^{15}N -enriched maize residues) in barley compared with that in vetch further supports the abovementioned higher potential of cereals for soil N uptake (De Notaris et al. 2020; Langelier et al. 2021; Guardia et al. 2025), likely due to the biological N fixation capacity of legumes. In the present experiment, the different cover cropping treatments had no significant effect on N recovery in maize plants from the preceding maize residues (Table 3). To date, no data are available regarding the effect of cover cropping on N recovery from mature crop residues in the subsequent cash crops. The average plant_NR_{15RES} of 7.7% is within the range reported by other studies. Mubarak et al. (2003) observed, in a maize–groundnut rotation, a 9% N recovery in groundnut, followed by a 2% N recovery in maize after maize residue recycling. Similarly, Ding et al. (2019) reported a 9.2% N recovery from maize residues in wheat in a pot experiment, whereas Taveira et al. (2020) reported a N recovery from aboveground and belowground biomass corn residues ranging from 9.7% to 13.5% (depending on tillage management) in soybean, highlighting the higher contribution of belowground biomass (which was not traced in our study) to N recovery by subsequent crops. Moreover, the relatively low N recovery from recalcitrant maize residues can be attributed to the greater availability of alternative N sources, such as synthetic fertilizer applied to maize and N from less mature cover crop residues (Liang et al. 2022), particularly those from legumes.

The average 33% maize plant N recovery from the synthetic fertilizer reported in this experiment (Fig. 6) was higher than the 17% and 21% reported by Taveira et al. (2020) and Mubarak et al. (2003), respectively, in maize croplands. We hypothesized that the lower availability of endogenous N (soil and cover crop residues) in barley-amended microplots may have increased the plant recovery of ^{15}N -labeled fertilizer compared with that in legume or fallow treatments (Fig. 6). Several factors may have contributed to the lower availability of soil NO_3^- in barley microplots

during the early maize development stages (Fig. S3b). These include the enhanced crop N uptake during the barley growing period, the expected lower mineralization and N supply from barley residues compared with those from vetch residues (Gabriel and Quemada 2011; Sullivan et al. 2019; Guardia et al. 2025), and the likely enhanced immobilization of mineral N caused by the higher C:N ratio of barley residues (Table 1) (McSwiney et al. 2010; Chen et al. 2014). It should be noted that this crop N recovery advantage, observed when maize was preceded by barley rather than vetch, was obtained under similar synthetic N application rates. In this sense, the benefits that legumes provide agro-ecosystems and society, including increments in soil organic C (30%) and reduction in the dependence of synthetic fertilizers (40%) must be considered in the design of future sustainable rotations (Jensen et al. 2012; Srivastava et al. 2025). Therefore, further research is required in the context of integrated fertilization strategies, ideally including combined ^{15}N labeling and microbial analyses (i.e., abundance of nitrifying abundances) to better elucidate the effect of residue quality on transformation rates of exogenous N.

The average 22% soil N recovery from the fertilizer (Fig. 6) was consistent with the 17%–29% range reported by Taveira et al. (2020) with maize at a soil depth of 0–45 cm. However, these recoveries were lower than those observed in rainfed crops, such as the 29%–50% N recovery in barley reported by García-Gutiérrez et al. (2023) and the 29% in wheat reported by H. Li et al. (2023a). This discrepancy could be attributed to the lower N leaching losses typically observed in rainfed semiarid croplands compared with irrigated systems. Regarding the comparison between cover cropping treatments, the soil N content derived from the ^{15}AN fertilizer (but not from A^{15}N) was higher in barley than in fallow and vetch (Table 4). Our results suggest that rotations with barley as the cover crop tend to increase NH_4^+ retention in soil while decreasing residual NO_3^- , particularly in the deeper soil layers (40–60 cm), where the leaching potential is higher during subsequent cropping phases. Further research is required to elucidate the physical and chemical mechanisms underlying this tendency.

Considering the fertilizer N recovery in both plant and soil (average 55%) and the 0.23% of fertilizer N lost via N_2O emissions, approximately 45% of the

applied N at fertilization remained unaccounted for. This suggests a critical influence of main quantitative N loss pathways (Sutton et al. 2013; Ussiri and Lal 2013), including denitrification-induced N_2 emissions (favoured under irrigated conditions, as reported by Guardia et al. (2018)), NH_3 volatilization (favoured in high pH soils as those of our study) (Pan et al. 2016) and N leaching. In this sense, cover crops and intercropping arise as effective strategies for mitigating leaching losses during the fallow period in irrigated semi-arid croplands (Gabriel et al. 2013; Thapa et al. 2018). Our results highlight the critical need to identify site-specific N loss pathways to optimize fertilizer efficiency and minimize N surpluses and associated environmental risks (Galloway et al. 2003; Lassaletta et al. 2014).

Conclusions

Using ^{15}N enrichment, this field experiment confirmed a low contribution of maize residue to N_2O emissions and N cycling in a cover crop–maize rotation under semiarid conditions, so the beneficial retention of maize residues does not involve short-term pollution swapping in terms of N_2O under the conditions of our study.

On average, 0.23% of the synthetic N applied was lost as N_2O , with 66% of these emissions derived from fertilizer NH_4^+-N , particularly during N_2O hot moments after maize fertilization. This supports the relevance of NH_4^+ oxidation processes (e.g., nitrification, nitrifier denitrification, or coupled nitrification–denitrification) over denitrification in semiarid cropping systems, even under irrigated conditions. This finding involves pivotal implications for the choice of right source in conventionally-managed (i.e., use of synthetic N fertilizers) irrigated rotations in Mediterranean areas. Due to the higher emissions under vetch cover crop application, it would be relevant to further study the relevant N transformation rates after cover crops incorporation, including the induced priming effect of biologically fixed N triggering soil mineralization.

Comparing the effects of different cover cropping treatments (legume, nonlegume, and bare fallow) indicated that the use of barley as a cover crop decreased total N_2O fluxes and increased total N

recovery (plant + soil), thus reinforcing the potential of cereal cover crops (pure stands or in mixtures) to enhance N recycling in irrigated semiarid rotations. However, this should be considered i) within the context of integrated soil fertility management, given the potential of legumes to decrease synthetic N inputs; ii) by accurately measuring changes in soil organic C stocks and other reactive N loss pathways to address potential side-effects and quantify the impact on net global warming potential.

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Author contributions Sandra García-Gutiérrez, Sonia García-Marco, Guillermo Guardia, Mónica Montoya and Antonio Vallejo conceptualized and designed this study. The data collection was done by Sandra García-Gutiérrez, Sonia García-Marco, Guillermo Guardia, Rafael Jiménez-Horcadada, Mónica Montoya and Rocío Rodríguez-Pérez. Sandra García-Gutiérrez, Sonia García-Marco, Guillermo Guardia and Rafael Jiménez-Horcadada performed the data analysis. The first draft of the manuscript and data visualization was done by Sandra García-Gutiérrez, with the supervision of Sonia García-Marco and Guillermo Guardia. Antonio Vallejo made substantial contribution to the final draft. All authors contributed to the review and editing of the final manuscript and approved the final manuscript.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

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