Plant Soil
https://doi.org/10.1007/s11104-026-08775-2

RESEARCH ARTICLE

Spontaneous vegetation monitoring as key soil bio-indicators in mangrove rice production agroecologies in Guinea Bissau

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Received: 20 February 2026 / Accepted: 16 June 2026

Abstract

Aims Mangrove rice production (MRP) is essential for food security and environmental sustainability among smallholder farmers in Guinea-Bissau. Distinct agroecologies within MRP systems are shaped by specific soil–water conditions and by the spontaneous vegetation (weeds) colonizing rice fields. This study evaluates the potential of dominated weed species as bio-indicators of soil fertility and organic matter dynamics across contrasting mangrove rice agroecologies.

Methods Approximately 300 soil samples were collected during 2022 and 2023 and analyzed for carbon (C) and nitrogen (N), and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). In parallel, 155 weed species were surveyed using random field trials (2022) and transect-based methods (2023). UAV multispectral imagery (Phantom 4) and random forest classification were applied to assess weed spatial distribution and species dominance.

Results Weed distribution followed a clear agroecological gradient, with *Echinochloa colona* dominating Associated Mangrove fields and *Blutaparon vermiculare* prevailing in Tidal Mangrove fields. UAV classification achieved up to 88% accuracy, although performance decreased in densely vegetated fields. Soil isotopic composition revealed distinct agroecological

Responsible Editor: Tida Ge.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-026-08775-2.

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Published online: 25 June 2026

patterns, with higher $\delta^{15}N$ values and C ratios in tidal mangrove agroesystems, suggesting enhanced nitrogen mineralization and faster organic matter turnover. $\delta^{13}C$ signatures reflected differences in organic matter sources and turnover dynamics between agroecologies. Isotopic analyses clearly distinguished C_3 and C_4 vegetation, while C_3 species showed significantly higher nitrogen content than C_4 species, indicating contrasting nutrient-use strategies.

Conclusions Spontaneous vegetation provides a robust proxy for assessing coupled carbon and nitrogen dynamics in mangrove rice systems. Integrating isotopic and spatial approaches enable effective discrimination of agroecological conditions, though further refinement is needed to align these tools with farmers' management practices.

Keywords Soil fertility indicators · $\delta^{13}C$ and $\delta^{15}N$ isotopes · Weed ecology · UAV-based monitoring and classification

Introduction

Rice cultivation is crucial for most West African countries as a primary food source. For small-scale farmers in Guinea-Bissau, maintaining a minimum production to feed their families is a daily struggle. Numerous changes, ranging from natural causes to anthropic factors such as the climate change to new socioeconomic trends and market instability, make these small-scale agricultural ecosystems highly vulnerable (Adeyeri et al. 2026).

The "Balantas" ethnic group of Guinea-Bissau is known as the "Mangrove Rice Specialists" because they were pioneers in the use and adaptation of rice cultivation techniques in the coastal mangrove areas of the country. The life of the Balanta people revolves around rice monoculture, not only as their main diet but also in the construction of houses (using straw remnants) and for feeding animals. Additionally, rice plays a central role in all social activities and many spiritual ceremonies (Temudo and Santos 2017).

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The "Bolanhas" (rice fields in Kriol) are traditionally managed and well-preserved agroecologies, where external soil fertilization practices are absent. In these areas, the incorporation of nitrogen (N) and phosphorus (P) takes place solely through the mineral hydrolysis of soils, decomposition and mineralization of organic matter, and dissolved/suspended materials in water inflows, whether through freshwater or the ingress of saltwater from tides. This process not only enhances other macro and micro soil nutrients but also contributes to good yields (Olk et al. 1996).

However, crop requirements for having good production are high, while the possibilities of applying fertilizers, whether synthetic or organic, are very limited in these agro-systems, meanwhile soil nutrient pools are decreasing (Merkohasanaj et al. 2025a; Garbanzo et al. 2025). Thus, it becomes crucial to have deeper knowledge and evidence of the nutritional flux that occurs in the soils of these regions and the role of carbon and nitrogen-fixing plants.

In mangrove swamp rice systems, agroecologies refer to distinct production environments within the landscape defined by interacting gradients of soil properties, hydrology, salinity, and topography, which jointly regulate plant performance and management practices. In Guinea-Bissau, these are commonly distinguished as Tidal Mangrove (TM) and Associated Mangrove (AM) systems, reflecting differences in water control and soil physicochemical conditions (Merkohasanaj et al. 2025a, b; Garbanzo et al. 2024) consistent with broader definitions of agroecological environments in West African rice systems (e.g., AfricaRice; Temudo and Santos 2017). Thus, these agroecological units represent functional soil–plant environments where biophysical constraints and farmer practices interact, shaping both rice productivity and vegetation dynamics.

Spontaneous green vegetation (weeds) refers to non-cultivated plant species that naturally establish in agricultural systems. Their composition and abundance reflect environmental conditions and soil properties, providing insight into underlying agroecosystem processes. Thus, they can act as effective bioindicators of soil fertility. Bioindicators integrate biological, chemical, and physical aspects of soil functioning and respond sensitively to variations in nutrient availability and organic matter dynamics. In this context, weed communities are closely linked to gradients in soil fertility, hydrology, and salinity,

providing a practical and integrative proxy for assessing soil quality in low-input systems (Carlesi and Barberi 2017; MacLaren et al. 2020).

Weeds constitutes one of the main organic matter sources—including carbon and nitrogen—in these agroecologies during the initial stage of rice production (soil preparation) (Rao et al. 2007). However, during later production stages, weeds may become one of the main constraints to rice production if not properly managed (Bastiaans et al. 2008). Inadequate land preparation (soil tillage, soil leveling), poor quality and contaminated rice seeds, inadequate water management, mono-cropping, labor shortages for hand weeding and delayed herbicide applications are some of the agronomic factors exacerbating weed problems (Alagbo et al. 2022; Martin et al. 2021; Rodenburg and Johnson 2009). In the upland and lowland rainfed rice systems, the input levels are generally low, and low yields are commonly (range: 0.1–3.5 t/ha) due to poor soil fertility and weed competition (Balasubramanian et al. 2007), while numbers indicate ranges of 28–74% in transplanted lowland rice systems in the West Africa (Johnson et al. 2004).

Each rice production system harbors weed species well adapted to the environment and management practices. While the weed flora of a specific production system (e.g., lowland or upland) may be similar across different agroecological zones, the abundance of individual species can differ substantially (Akobundu and Fagade 1978).

The isotopic signal of nitrogen in the primary production of vegetal biomass offers a crucial perspective on biogeochemical processes influencing nutrient dynamics. The 15N/14N ratios of nitrogen-fixing plants compared to non-nitrogen-fixing ones have a significant differentiation (Mariotti et al. 1981; Amundson et al. 2003; Peterson and Fry 1987; Craine et al. 2009). This isotopic approach provides a valuable tool for understanding the relative contribution of nitrogen sources and their effects on primary productivity, thereby contributing to the sustainable management of farmland and grassland ecosystems. The natural abundance of N isotopes in soils and plants is also correlated with environmental variables, most importantly climate, at both local and global scales (Wang et al. 2014). As shown by Amundson et al. (2003), wetter and colder ecosystems appear to be more efficient in conserving and recycling mineral N. The use of plant $\delta^{15}N$ values to estimate the proportion of biologically fixed nitrogen in plant tissues (Shearer and Kohl 1988) is widely applied in ecology and agriculture. This approach originated from early studies that used ^{15}N to trace the fate of agricultural nitrogen in the environment (Kohl et al. 1973).

Carbon export and storage processes in wetlands are reflected in soil characteristics. Where sedimentation is considerable and marine connectivity is strong, phytoplankton and seagrass detritus will have a significant contribution to the soil C (Kristensen et al. 2008). Such processes commonly occur in estuaries and coastal mangrove ecosystems. In addition to marine-derived organic matter, the isotopic composition of soil carbon is also influenced by the surrounding vegetation types. In warm regions such as the studied area, plants with C3, C4, and CAM photosynthetic pathways may naturally coexist (Teeri and Stowe 1976; Cerling 1984). These plant groups differ in their carbon isotopic signatures, with C3 plants tissues generally exhibiting more negative $\delta^{13}C$ values due to the greater isotopic fractionation of ^{13}C by the enzyme RuBisCo during CO_2 assimilation (Bender 1971). In contrast, C4 plants, regulated by the enzyme PEP carboxylase, show less negative values as they fractionate to a lesser extent. CAM plants are characterized by higher isotopic variability (O'Leary 1981). These $\delta^{13}C$ isotopic signals in soil organic matter serve as reliable indicators of the dominant vegetation type and thus reflect the composition of organic inputs into the soil (Cerling 1984; Ehleringer et al. 1997; Andreetta et al. 2016).

Beyond vegetation type, the water use efficiency (WUE) of plants and other physiological stress factors (e.g., drought, heavy metal contamination) also affect carbon isotope discrimination. These conditions typically lead to stomatal closure, resulting in reduced carbon fractionation and higher $\delta^{13}C$ values (Domergue et al. 2022; Farquhar et al. 1982; Pate 2001). Carbon isotopic values are influenced by physiological and environmental stress factors such as drought or heavy metals presence, which induce stomatal closure and reduce carbon fractionation (Wang et al. 2022; Zhao et al. 2004). As a result, $\delta^{13}C$ variations serve as an integrated indicator of plant responses to environmental stress, including water limitation and Fe-related contamination.

In parallel, nitrogen isotope ratios ($\delta^{15}N$) provide complementary information about nitrogen sources

and cycling. Together, $\delta^{13}C$ and $\delta^{15}N$ values offer a powerful tool to trace environmental conditions, crop responses, and yields across different years and microenvironments. This isotopic traceability can be linked to site-specific factors such as soil properties, crop rotation patterns and water availability (Domergue et al. 2022; Wang et al. 2022; Zhao et al. 2004; Shearer and Kohl 1988; Peterson and Fry 1987).

Remote sensing (RS) and unmanned aerial vehicles (UAVs) have emerged as valuable tools for weed management in agriculture. These technologies enable efficient detection, mapping, and monitoring of weed infestations, supporting site-specific management strategies (Shaw 2005; Roslim et al. 2021). RS techniques, including hyperspectral, multispectral, and RGB sensors, can effectively assess weed spatial distribution, abundance, and density (Roslim et al. 2021). Recent studies have demonstrated the efficacy of drone-based herbicide applications, evaluated using RS data such as reflectance and vegetation indices (Bautista et al. 2024). Moreover, the integration of plant functional traits in RS analysis enhances the discrimination between invasive and native species, with phenological and structural traits being well-exploited, while physiological traits remain underutilized (Niphadkar and Nagendra 2016). These advancements in RS and UAV technologies offer promising solutions for addressing future challenges in weed management, including food security, sustainability, and herbicide resistance (Roslim et al. 2021).

Building upon this background and local farmers´ knowledge of the different weed species and their management, this study aimed to identify and map the most abundant weed species across the different agroecological systems. We further investigate their spatial distribution, isotopic characteristics, and contribution to soil nutrient pools, with the overarching goal of improving rice production while ensuring the agroecological conservation of these traditional systems.

Despite the recognized importance of spontaneous vegetation in low-input rice systems, its role as an integrated bio-indicator of soil fertility and soil organic matter dynamics in mangrove rice production systems remains insufficiently characterized. Previous studies have described vegetation patterns, soil properties, or isotopic signatures separately, but few have combined field-based vegetation surveys, stable isotope analyses, and UAV-derived spatial data to evaluate whether dominant spontaneous vegetation species can provide reliable information on underlying soil processes.

We hypothesized that (i) spontaneous vegetation composition, abundance, and isotopic signatures would vary consistently along the main agroecological gradients of mangrove rice systems, reflecting differences in hydrology, salinity, and soil fertility; and that (ii) UAV-based multispectral imagery would be able to identify and map the dominant vegetation patterns associated with these gradients. Accordingly, the objectives of this study were to identify the dominant spontaneous vegetation species across the main agroecological zones, evaluate their relationships with soil carbon and nitrogen dynamics, and assess the utility of UAV-based classification for mapping vegetation patterns relevant to soil management.

Material and methods

Study area characterization

This research is concentrated in two main coastal regions of Guinea Bissau, Oio (central) in the villages of Malafu end Enchugal and Tombali (south) in the villages of Cafine and Cafal (Fig. 3). The major difference between the two regions is the different precipitation regime, which influences the abundance of the different plant species populations in each region. Farmers adapt their farming and production practices according to their agroecology conditions, which brings a high diversity of labour, planting and transplanting practices and selection of rice varieties. In general, farmers plough their lands in plots (locally referred as ¨priks¨ in Kriol), which represent farmer-defined field divisions. The plots vary in size, typically ranging from small (300 m^2) areas to larger parcels (1000 m^2) depending on local conditions and management practices. Plot preparation occurs in two phases/periods: the first one starts when the soil is quite humid (after the first rains), they overturn the left rice straw from the last year and the present spontaneous green vegetation at the bottom of what will be the new ridges. In the second phase, they raise the ridges to an average high of 40 cm, taking the soil on both sides of the ride. From now on, we will

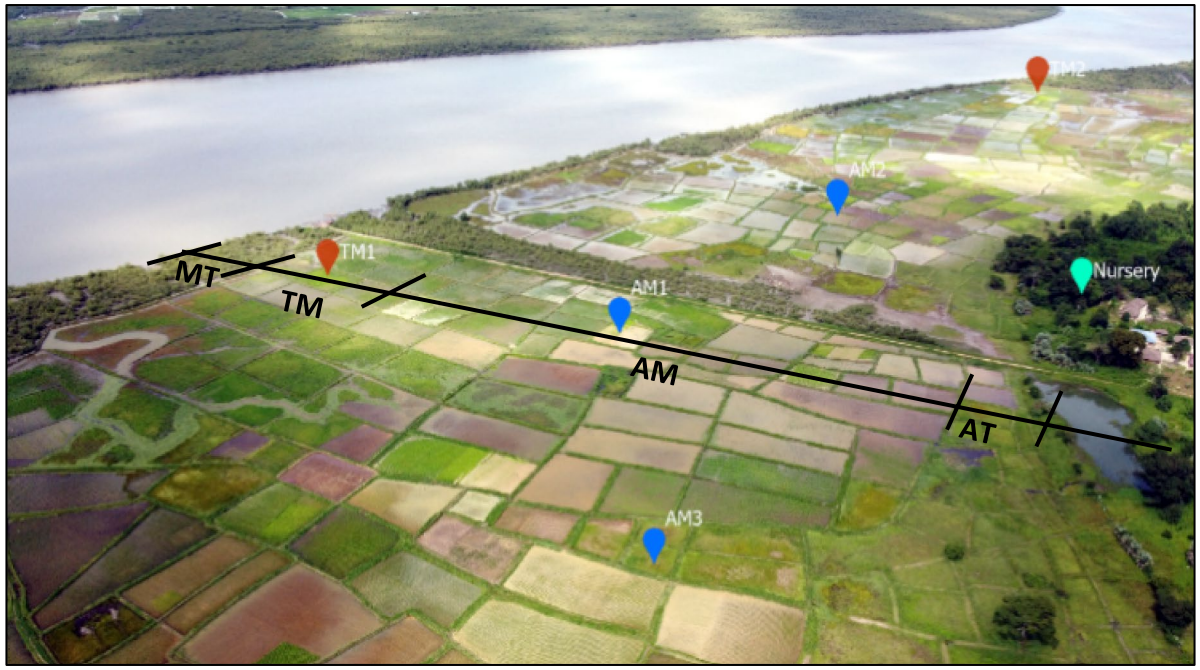

Fig. 1 Schematic cross-section of the catena showing the spatial distribution of rice agroecologies in the study area (Cafine village, Tombali, Guinea Bissau). Four main agroecologies are represented: Nurseries- in the upper part close to the village; Associated Terrace (AT)—the first rice fields close to the nurseries; Associated Mangrove (AM) – rainfeed rice fields; Tidal Mangrove (TM) – tide influenced rice fields, and Mangrove Terrace (MT)—mangrove forests close to the river

refer to these spontaneous green vegetation or plant species as ¨weed¨. Schematic cross-section of the catena (Fig. 1) shows four predominant divisions and rice field terrace sub-divided into three main agroecologies as follows: AT—Associated Terrace; AM—Associated Mangrove; TM—Tidal Mangrove and MT – Mangrove Terrace. Being close to the residual and the intermediate terrace, the AT fields occupy a very small part (less than 10%) and are the last ones used for rice cultivation, if required. Downstream runoff and unsustainable water management are the reason for the abandonment of these fields, used then only for pasture. The AM fields occupy 70—80% of the rice cultivation fields, characterized by low rides and sometimes limited drainage and water shortage. AMs are mostly loamy and clay-loamy soils, flooded by runoff and precipitations, and mainly dominated by Poaceae species as the *Echinochloa colona* (¨keu¨ in Balanta) and less abundant *Cyperus esculentus* (Cyperaceaes family, miu-miu in Balanta) (Fig. 2c, d).

The TM are the preferred fields for farmers due to more favorable soil and water management condition They occupy just 20% of the total cultivation space since they have favorable soil properties due to the influence of tides, with sufficient water drainage. However, under water shortage conditions, some TMs suffer severe problems of salinization (Van Gent and Ukkerman 1993). In TMs particular soil conditions with very high clay content and salinity intrusion, two main plant species were observed: *Blutaparon vermiculare* (Amaranthaceae family) and *Sesuvium portulacastrum* (Aizoaceae family). Commonly, farmers called them by the same name ¨malugreta¨ (Balanta local name) (Fig. 2 a, b). In the mangrove terrace—MT (Fig. 1), we observe the prevailing mangrove species of *Rhizophora* and *Avicennia* (Van Gent and Ukkerman 1993).

Sampling methodology

Soil sampling

During the 2022 and 2023 rice growing seasons, respectively a total of 122 and 175 topsoil samples

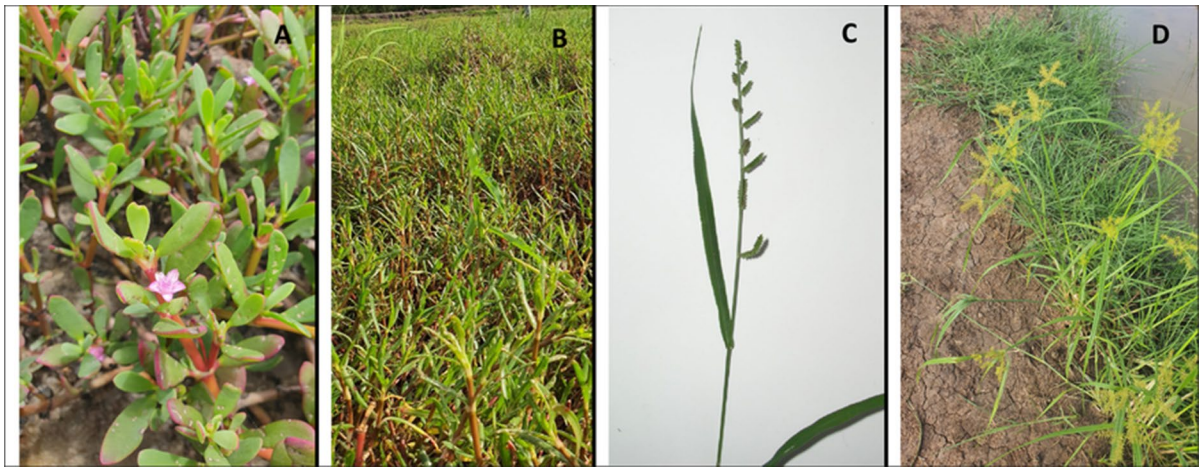

Fig. 2 Main weed species identified in the TM and AM agro-ecologies. **A**. *Sesuvium portulacastrum* (found in TM plots); **B**. *Blutaparon vermiculare* (also found in TM plots); **C**. *Echinochloa colona* (found in AM plots); **D**. *Cyperus esculentus* (found in AM plots mainly located in the separation edges between plots)

(0–20 cm depth, composite of three replications) were collected respectively in Oio region—village of Enchugal and Malafu, and Tombali region—village of Cafine and Cafale. Sampling was conducted at two key phenological stages for rice cultivation: (a) at sowing or transplantation, occurring approximately 2–3 weeks after plowing; and (b) at the stage of flowering or grain formation, which typically occurs 4–6 weeks after transplantation; Both periods represent critical phases in the rice growth cycle when nutrient demand is high. In both agro-ecological zones, soil samples were collected using a randomized spatial distribution approach (Fig. 1, refer to Merkohasanaj et al. 2025b for more details

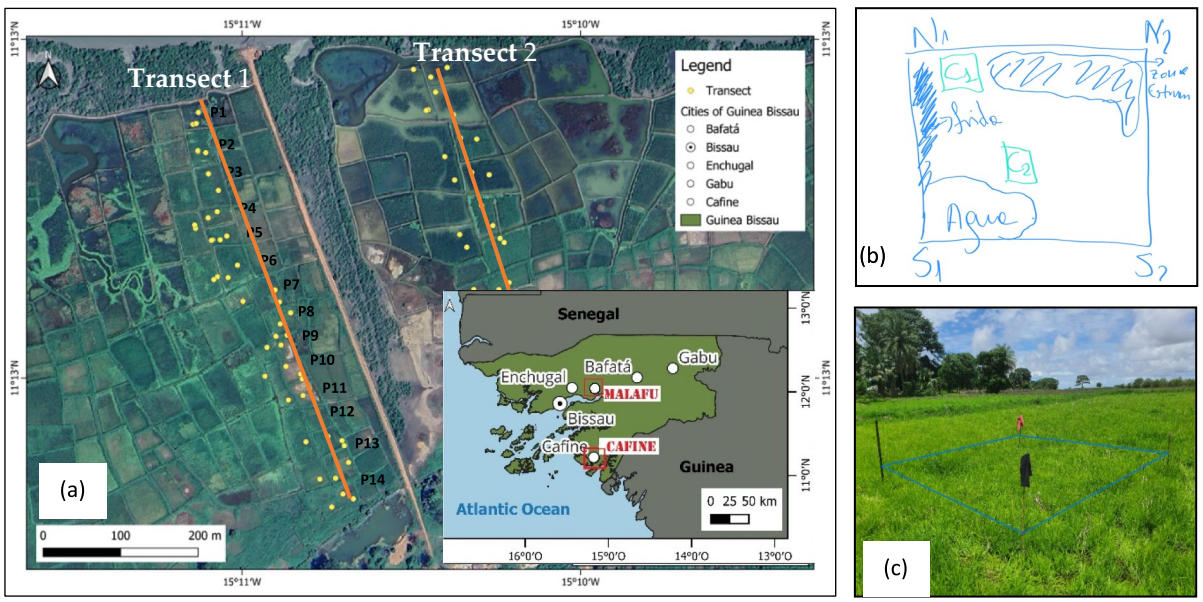

Fig. 3 (**a**) Location of the transects for the Cafine village (P = plots, Transect 1 length = 550 m, Transect 2 length = 450 m) and regional location for Tombali – Cafine village and Oio- Malafu village in red; (**b**) design and field notes for each plot and two grids (C1 & C2) used as validation points; (**c**) grid designed as a square of 5 by 5 m—July 2023

on sampling, storage and analytical methodologies), ensuring broad and representative coverage of the study areas. The samples were subsequently air-dried, sieved, and prepared for shipment in the Soil and Water laboratory of Bissau before being transported to the Universidad de Granada Soil Laboratory and the Instituto Andaluz de Ciencias de la Tierra Stable Isotope Biogeochemistry Laboratory for further analyses.

Weed sampling

For 2022, 54 plant samples were collected in the same plots where soil samples were taken and for the same sampling periods.

Then for 2023, in order to enhance the accuracy of estimating the spatial distribution of the different weed species within the plots, we conducted "field transects" in two of the four villages (Malafu—Oio and Cafine—Tombali) (Fig. 3a). Transects involved identifying and collecting all weed species present within each plot, while then two grid per plot were used to qualitatively define their abundance, density and coverage in each plot (Fig. 3b,c). For the identification of different species, we used the botanical guide *Flora infestante das culturas de bolanha da Guiné-Bissau* (Moreira and Martins 2002). In total, 100 weed species were sampled, stored, codified according to the plot and grid number, and then air dried and prepared for laboratory analyses.

Particulate Organic Matter (POM) sampling

Using a filtration setup consisting of a 60 mL syringe and a 20 mm diameter of silica filter, we collected 47 filtered water samples during the 2022 campaign. The standard procedure involved filtering up to five syringes per sample (i.e., a maximum of 300 mL of water per sample). After filtration, the filters were carefully removed to prevent contamination, air-dried, coded, and stored in aluminum foil for preservation and further laboratory analysis. In 2025, an additional 16 filtered water samples were collected from nearby river flows and remain water in the rice field channels, choosing the ones maximum possible near to the fields sampled in 2022.

Weed spatial distribution and image processing

A Phantom 4 multispectral drone (Ph-4) was used to identify and estimate the distribution and abundance of the different weed species, making the identification the closer possible to the highest vegetative state before farmers work the fields and incorporate the plants into the soil for both years (2022 and 2023).

Drone flights (Phantom 4) were planned at 100 m height, covering the entire distribution of the selected transects (as explained in weed sampling section). The drone flights and the field transects took place on the same date as the planned in order to capture the green vegetation at its peak growth stage, both conducted before farmers plough their fields.

Multispectral images were then processed using Agisoft Metashape software (http://www.agisoft.com) to generate orthorectified images and digital surface models (DSM). A complex machine learning process was used to calculate with the bands RGB, NIR, RedEdge, and different vegetation indexes (Table 1) to get a final Random Forest Classification, practically separating the green biomass into main classes as: Species of Interest (predominant species), Water, Bare Soil, and others (other secondary species with very little abundance). This automatic learning process was performed in the Google Colaboratory (https://colab.research.google.com/) programming environment, using Python programming language, to avoid computational burdens on local computers. Further image acquisition timing, parameterization and classification processing are detailed in Annex I.

Table 1 Vegetation indexes used as co-variables

Index	Equation	Reference
Normalized Difference Vegetation Index (NDVI)	(NIR − Red)/(NIR + Red)	Rouse et al. (1974)
Normalized Difference Red Edge (NDRE)	(NIR − RedEdge)/(NIR + RedEdge)	Tucker (1979)
Simple Ratio (SR)	(NIR/Red)	Jordan (1969)
Soil Adjusted Vegetation Index (SAVI)	1.5 ((NIR − Red)/(NIR + Red + 0.5))	Huete (1988)

Soil, weeds and water analyses

Soil analyses were conducted in the Soil and Agricultural Chemistry Laboratory at Granada University (UGR) for pH (in water (pH_{H2O}), Electrical Conductivity (EC) and Redox potential (1:5 soil–water), texture with the Bouyoucos method, Organic Matter (OM) were measured by dichromate oxidation with Tyurin method, P and K by Egner-Richm method and Cation Exchange Capacity (CEC) were extracted with ammonium acetate and measured by Atomic Absorption Spectrophotometry. Check Merkohasanaj et al. 2025b for used methodologies.

Furthermore, isotope measurements were carried out at the Stable Isotope Biogeochemistry Laboratory of the Instituto Andaluz de Ciencias de la Tierra (CSIC-UGR, Granada), for the soil, plant tissues (both leave and stems), and POM (Particulate Organic Matter) in water samples (extracted by the filters) was analyzed for the isotopic composition of nitrogen and carbon by means of a Carlo Elba NC1500 (Milan, Italy) elemental analyzer on line with a Delta Plus XP (ThermoQuest, Bremen, Germany) mass spectrometer (EA-IRMS). The stable composition is reported as δ values per mil:

$$\delta = (Rsample/Rstandard - 1) * 1000$$

where $R = {}^{13}C/{}^{12}C$ for $\delta^{13}C$ values and $R = {}^{15}N/{}^{14}N$ for $\delta^{15}N$.

Commercial CO_2 and N_2 were used as the internal standard for carbon and nitrogen isotopic analyses. For carbon 22 internal standard (organic and inorganic material) ranging between −49.44 ‰ to + 28.59 ‰ (V-PDB), contrasted with the IAEA international references NBS-28, NBS-29, NBS-20 (carbonates) and NBS-22, IAEA-CH-7, IAEA-CH-6 (organic material), are used in relation to the isotopic range of samples to be analyzed. For this study, 2 internal standards of −30.63‰ and −11.65 ‰ (V-PDB) have been used. For nitrogen, 9 internal standards (organic and inorganic material) ranging between −1.94 ‰ to + 16.01 ‰ (AIR), contrasted with the IAEA international references IAEA-N-1, IAEA-N-2, NO-3, USGS32, USGS34 and USGS35. For this study, 2 internal standards of −1.02‰ and + 16.01 ‰ (AIR) have been used.

Precision calculated, after correction of the mass spectrometer daily drift, from standards systematically interspersed in analytical batches was better than ± 0.1‰ for $\delta^{15}N$ and $\delta^{13}C$. The standard for reporting carbon measurements is V-PDB (Vienna-PDB) and for nitrogen measurements is atmospheric nitrogen (AIR).

Statistical analysis

Descriptive statistics (minimum, maximum, mean and standard deviation) were calculated for all soil and plant variables, including isotopic signatures ($\delta^{15}N$, $\delta^{13}C$), elemental composition (%C, %N), and C:N ratios, for both sampling years (2022 and 2023). Prior to statistical analyses, data distributions and model assumptions were evaluated. Although some variables met the normality assumption while others did not according to the Shapiro–Wilk test, most deviations from normality were relatively smaller. For homogeneity and residual distribution, Levene's test indicated that variances were generally satisfied across groups, and sample sizes were relatively balanced. Thus, differences between agroecological systems (TM and AM), years, and plant functional types (C_3 and C_4) were assessed using analysis of variance (ANOVA). For soils, two-way ANOVA was applied with agroecology and year as fixed factors. For weed isotopic data, one-way ANOVA was used to test differences between C_3 and C_4 species, and two-way ANOVA was further performed to evaluate the effects of agroecology, year, plant type, and their interactions. Statistical significance was determined at $p < 0.05$. Relationships among soil and plants variables (%C, %N, C:N, $\delta^{15}N$, $\delta^{13}C$) were explored using Sperman correlation coefficients, and results were visualized through correlation heatmaps.

To investigate multivariate patterns and identify the main drivers of variability between soils and plants, a principal component analysis (PCA) was conducted using standardized variables. The PCA biplot was used to visualize clustering according to agroecological systems (AM and TM) and plant functional types (C_3 and C_4), as well as to interpret the contribution of isotopic and elemental variables to sample differentiation. All statistical analyses and graphical representations were performed using Python, with appropriate packages for multivariate analysis and visualization (Scipy, Statesmodels, Sklearn, MatPlotLib).

Table 2 Descriptive statistics of soil samples (n = 174) variables collected in the 2023 essay in the three agroecologies

	Sand (%)	Silt (%)	Clay (%)	pH (1:2.5)	EC (dS m^{-1})	OM (%)	C/N	C (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	CEC (cmol kg^{-1})
Nurseries (n = 38)												
mean	**66.9**	**15.0**	**17.8**	**6.7**	**0.5**	**2.7**	**13.5**	**1.2**	**0.1**	**3.5**	**106.3**	**6.7**
min	60.0	10.0	8.0	5.9	0.3	1.2	11.3	0.6	0.1	0.0	42.6	4.1
max	82.0	20.0	22.0	7.3	2.7	4.1	15.9	2.4	0.2	7.0	252.3	11.3
SD	4.7	5.8	4.4	0.5	0.3	1.8	2.6	1.0	0.08	1.6	184.7	4.7
AM—Associated Mangrove (n = 68)												
mean	**5.8**	**35.1**	**59.1**	**6.2**	**0.6**	**2.8**	**13.2**	**1.5**	**0.1**	**9.4**	**310.6**	**22.3**
min	1.0	18.0	42.0	5.2	0.1	1.4	10.9	0.9	0.1	0.0	123.1	12.2
max	15.0	47.0	79.0	7.4	1.6	5.8	15.8	2.7	0.2	47.7	511.7	48.7
SD	7.9	12.0	11.6	0.5	0.3	2.1	2.8	1.2	0.07	8.6	193.2	8.7
TM- Tidal Mangrove (n = 68)												
mean	**2.0**	**31.1**	**66.8**	**6.8**	**1.6**	**2.5**	**11.5**	**1.3**	**0.1**	**38.7**	**728.6**	**25.9**
min	0.0	16.0	44.0	6.1	0.2	1.0	8.8	0.7	0.1	0.0	287.8	5.1
max	9.0	48.0	82.0	7.4	5.7	4.3	14.0	2.6	0.3	91.5	1059.1	51.6
SD	10.5	14.0	15.1	0.7	1.5	1.8	2.5	0.8	0.06	22.2	253.5	9.5

Results

Soil and weed spatial characterization

Soil agroecological characterization

The results of soil analyses from the three identified agroecologies are presented in Table 2. TM soils exhibit a better fertility status, particularly in the availability of macro-nutrients (N-P-K). High salinity concentrations in these soils are generally not problematic since they typically decrease to acceptable levels for normal plant growth due to sufficient precipitation and drainage. In comparison to TM soils, AM agroecologies show lower organic matter (OM) and carbon (C) pools, measuring 2.8 versus 2.5 and 1.5 versus 1.3, respectively. However, the slightly acidic conditions with mean pH around 6.2, sometimes decreasing below 5.5, and lower N-P-K levels make AM soils less productive.

Monitoring weed spatial distribution and abundance

The results of image classification (Figs. 4 and 5) demonstrate a high level of accuracy in identifying and classifying primary plant species across both small plots and larger areas. The classification outputs show clear differentiation between major land cover classes, including water, vegetation, and bare soil. Furthermore, the classification results confirm the feasibility of identifying the three predominant species—*Blutaparon vermiculare*, *Sesuvium portulacastrum*, and *Echinochloa* spp.—with an accuracy of 70.9% (Fig. 4—Plot 1). In densely vegetated plots where *Echinochloa* spp. is dominant, misclassification occurs, particularly with other Poaceae species, especially when they share similar phenological stages (Fig. 5—Plot 4). The classification accuracy for these plots was recorded at 88%. While these results are promising, further improvements in the classification process, particularly through additional validation, are necessary to enhance overall accuracy.

The classification from the first transect done in Cafine, which include un area of approximately 10 ha, demonstrated that various vegetation indices are highly effective in capturing spatial variability across the landscape. Notably, the NDVI and SAVI indices (Fig. 6a,b) successfully delineated areas with dense or vigorous vegetation cover, while also identifying zones where undesirable weed growth occurs—providing farmers with actionable insights for targeted weed management. Moreover, the SR index, in conjunction with true-color imagery (Fig. 6c,d), proved reliable in detecting areas with elevated soil moisture

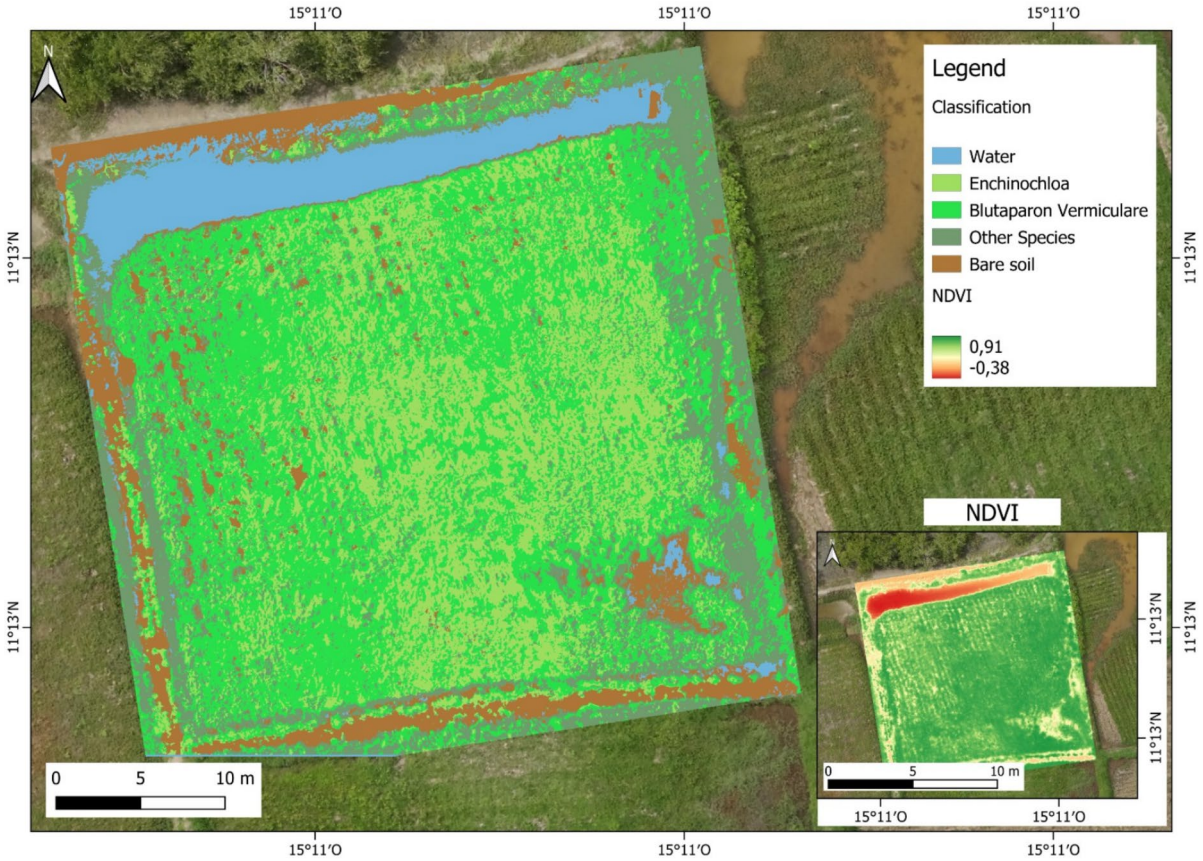

Fig. 4 Plot 1 final classification for two main identified plant species; Ph-4 image taken the 24th July 2023

and surface water, as well as zones affected by soil-related issues such as acidity, salinity, or toxicity — areas often appearing as bare patches with distinct soil coloration, resulting from ongoing soil mineralization processes. Integrating soil moisture data with the spatial distribution of specific weed species further enhances understanding of their physiological behavior, which is crucial for informing effective and site-specific weed management strategies.

Furthermore, plot-level composition derived from the UAV classification reinforces the field-based evidence of spatial heterogeneity in spontaneous vegetation along the transect (Fig. 1 in Annex II). The dominance of *'Echinochloa colona'* in several intermediate prikes, while the higher relative contribution of *'Blutaparon vermiculare'* in the first prike reflects the influence of tidal mangrove (TM) conditions, where salinity-tolerant species are more frequent. The increasing contribution of *'Other species'* in the upper prikes suggests a transition toward more heterogeneous vegetation assemblages, probably associated with local differences in soil moisture, drainage, management history, or proximity to less soil salinity conditions of the AM areas. Thus, the UAV classification did not only provide a map of dominant vegetation classes but also captured within-transect variation relevant for interpreting weed communities as spatial indicators of agroecological conditions.

Plant density effects

Transect field identification revealed a clear spatial gradient weed distribution (Fig. 7A). *Enchinochloa colona* predominated in the majority of the plots starting from P3 to the P11, mainly within the AM agroecology, whereas the first plots P1 and P2, located in the TM agroecology and more affected by saline intrusion, weredominated by salt-tolerant halophytes

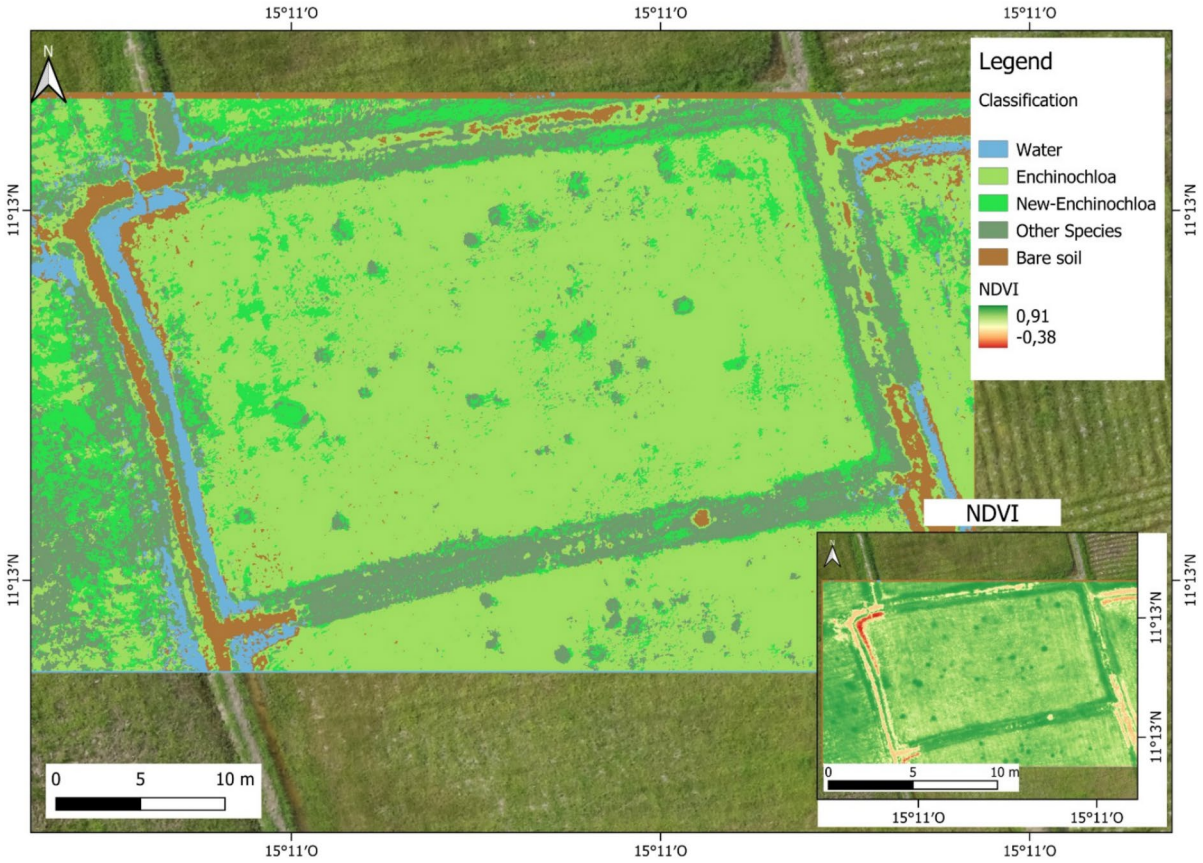

Fig. 5 Plot 4 final classification for the main identified plant species; Ph-4 image taken on 24th July 2023

species such as the *Blutaparon vermiculare* and *Sesuvium portulacastrum.* These species thrive in high-salinity environments—*Blutaparon portulacoides* tolerates hypersaline tidal flat conditions, while *Sesuvium. portulacastrum* grows well in full seawater (Duarte et al. 2013; Lokhande et al. 2011). Other less abundant species communities: Ancanthaces *Hypophila auriculata (frida)*, Cyperaceae such as *Cyperus tenuiculmis* (miumiu), *Cyperus articularus* (mampufa), *Eleocharis acutangulastopf* (mburu), and other Poaceae such *as Paspalium vaginatium* (ncada), *Paspalium scrobiculatum* (nconra) and *Acroceras amplectens stapf* (Ntchobandale) were more frequent in the upper plots (P13 and P14) near to the uplands ATs (see Fig. 1 to refer to the transect position and Fig. 2 for the plant communities).

The validated UAV classification showed clear spatial variation in vegetation composition along the transect (Fig. 7B). *Echinochloa colona* was the dominant class in several plots, particularly P3, P5, and P8, while *Blutaparon vermiculare* was more abundant in P1 and remained present throughout the transect. "New *Echinochloa colona*" was mainly associated with lower-numbered plots, whereas "Other species" increased in the upper plots, especially P12 and P14. Overall, the UAV classification was consistent with field observations and captured the main vegetation patterns across the agroecological gradient.

Furthermore, the UAV density classification highlights clear differences in vegetation abundance and density patterns across the agroecological gradient. Plot 1 (TM) shows a greater proportion of medium and high vegetation density classes, indicating denser and more continuous vegetation cover. In contrast, Plot 5 and Plot 8 (AMs) are dominated by bare soil and low-density vegetation, reflecting sparser biomass distribution (Fig. 8). These differences suggest

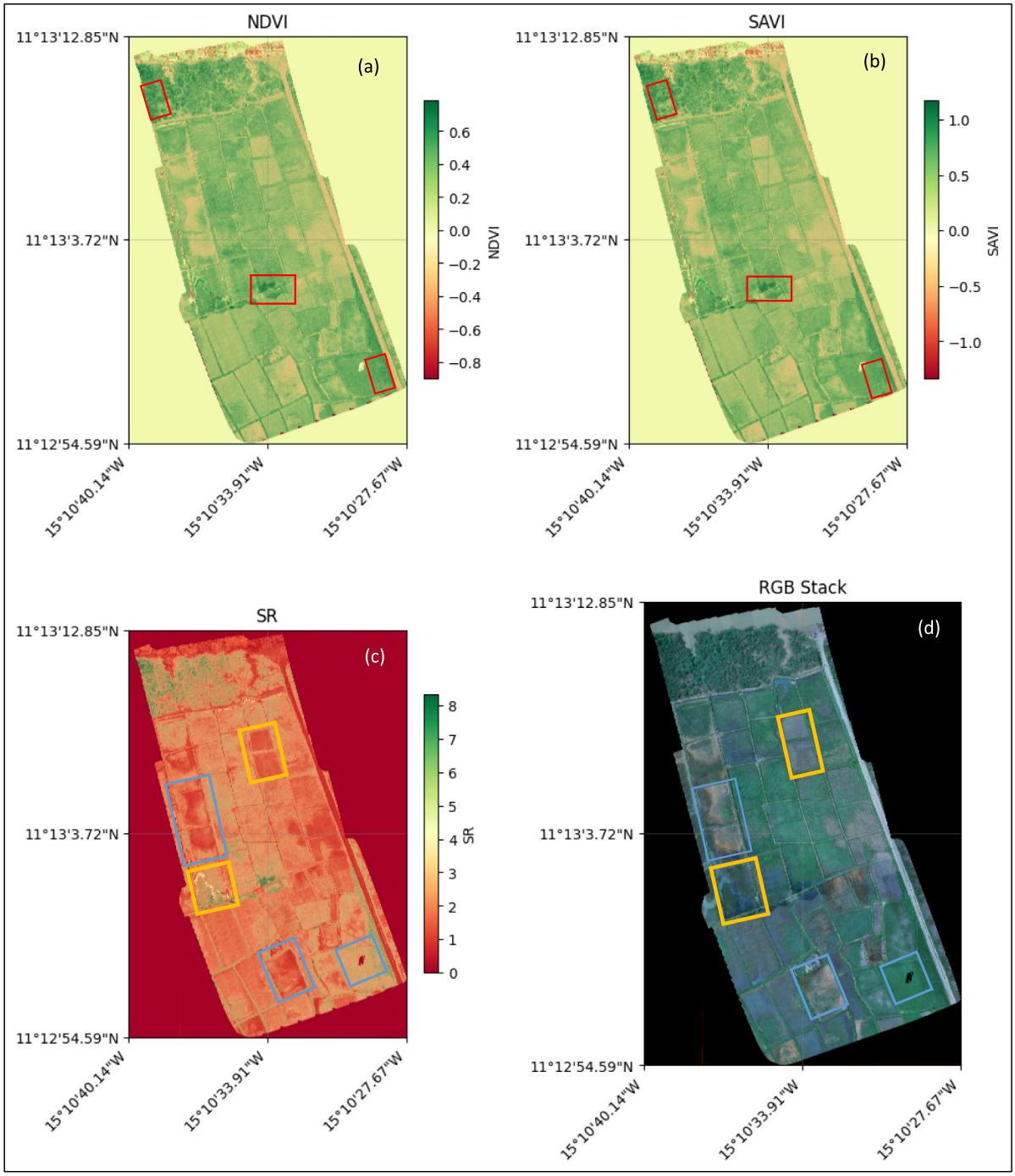

Fig. 6 Overall classification of Transect 1 and surrounding fields in Cafine village – July 2023. The red rectangles highlight areas of very high vegetation density as detected by the **a**) NDVI and **b**) SAVI indices. The blue and yellow rectangles indicate, respectively, zones of bare soil and high water content, as identified in the **c)** SR index and **d)** true-color (RGB) image

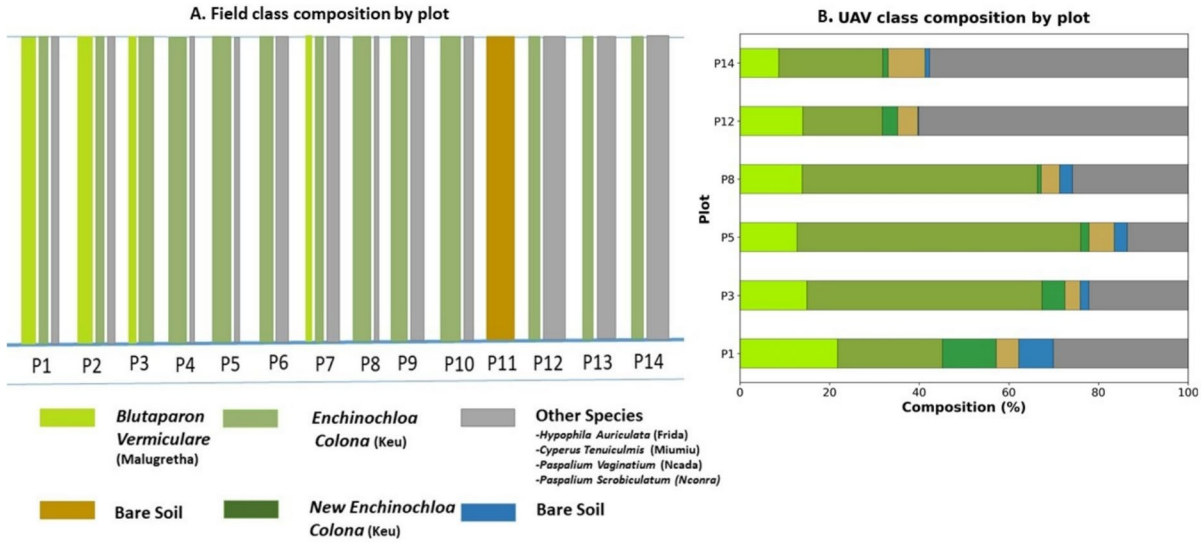

Fig. 7 **A**. Field classes distribution of the main identified species in each plot (P1-P14) along the transect 1 (Cafine village); P11 was ploughed few days before ploughed by the farmer, so weed identification was not possible for this plot **B**. Class composition by plot derived from the UAV-based Random Forest classification. Bars show the percentage of each classified vegetation or surface-cover class within each plot. Note: local names are given within brackets

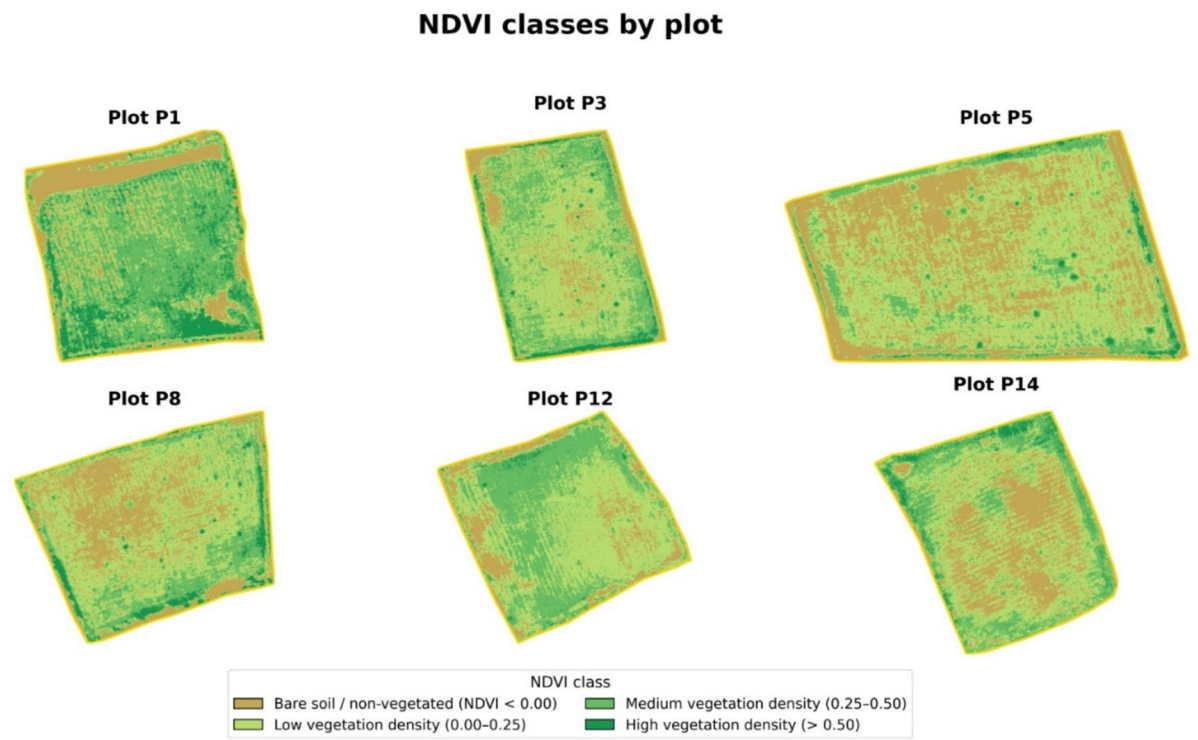

Fig. 8 NDVI-based vegetation density classification across plots along the agroecological gradient, showing denser vegetation in Plot P1 (TM) and lower vegetation densities in Plots P5 and P8 (AMs)

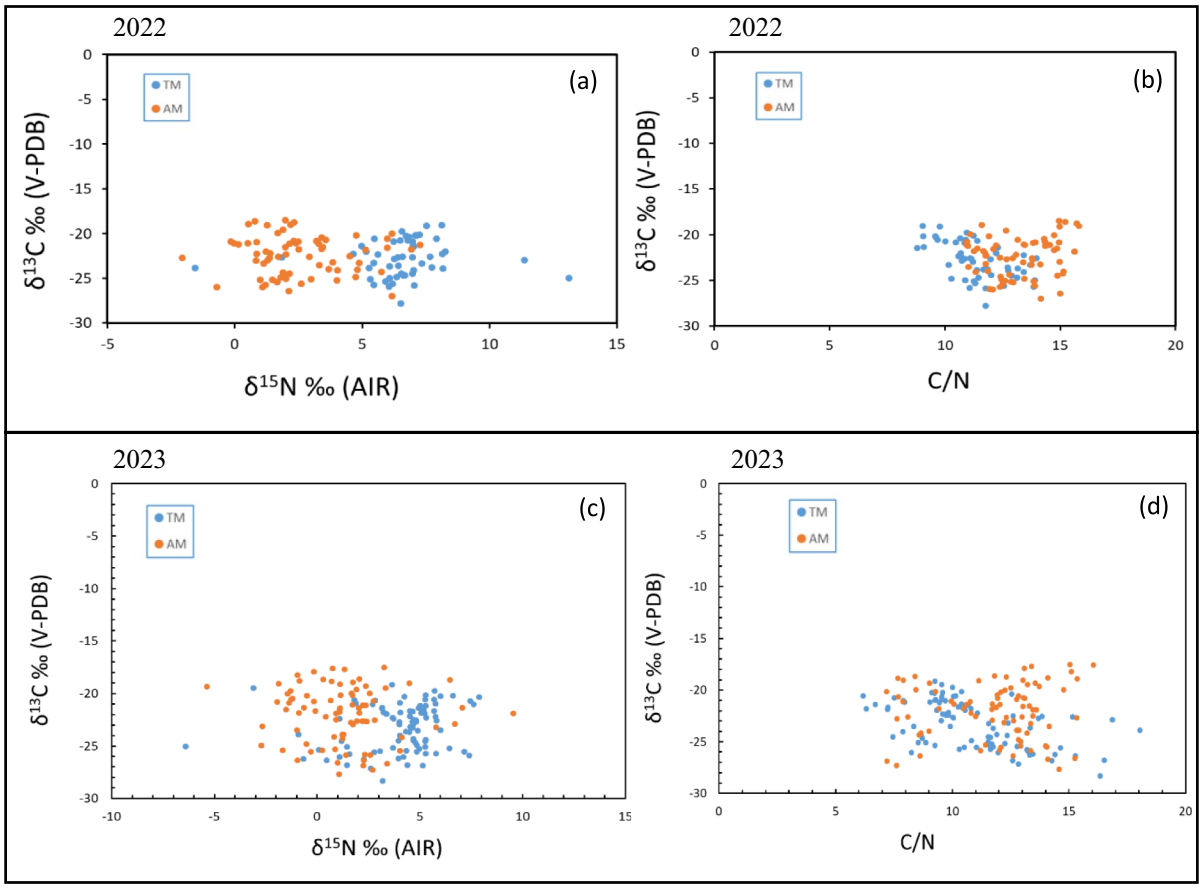

Fig. 9 (**a**) Isotopic $\delta^{15}N$ versus $\delta^{13}C$ and (**b**) C/N in relation to $\delta^{13}C$ for TM (Tidal Mangrove) and AM (Associated Mangrove) 2022 soil agroecologies (n = 122); (**c**) isotopic $\delta^{15}N$ versus $\delta^{13}C$ and (**d**) C/N in relation to $\delta^{13}C$ for TM (Tidal Mangrove) and AM (Associated Mangrove) 2023 soil agroecologies (n = 174).

contrasting vegetation productivity and organic matter inputs between agroecologies, which may partly explain the distinct carbon sources and turnover dynamics observed in TM and AM systems.

Soil and weeds isotopic signals

Soil isotops

Isotopic analyses of soil N^{15} signals across agroecological systems revealed clear differences between TM and AM soils. TM soils exhibit broader isotopic range, with higher mean $\delta^{15}N$ value of approximately 6.5 and 4 for 2022 and 2023 respectively (Fig. 9a,c; Table 3). In contrast, AM soils display a narrower isotopic range and lower mean $\delta^{15}N$ values (2.59 in 2022 and 1.2 in 2023) supporting the significant effect of agroecology observed with ANOVA (Table 4). For $\delta^{13}C$ isotopic, although the overall ranges were similar across agroecologies and years (−18‰ to −30‰ in 2022 and −17‰ to −28‰ in 2023) (Table 3), mean values differed markedly between systems. In 2022, TM soils exhibit higher less negative $\delta^{13}C$ values (−19.6‰) compared to AM soils (−25.3‰) (Fig. 9a), indicating distinct carbon sources or turnover dynamics between agroecologies. Soil carbon content was slightly higher in AM soils across both years. Differences in C/N ration were evident in 2022, with AM soils predominantly ranging between 13 and 15, while the majority of TM soils present C/N ratio between 8 and 12

Table 3 Descriptive statistics for 122 (TM and AM) soils sampled during the field campaign of 2022 and 174 sampled during 2023

Desc Statistics	d^{15}N ‰	d^{13}C ‰	C/N	%N	%C
TM – 2022					
Mean	6.55	−22.74	11.43	0.15	1.31
Max	13.12	−19.04	15.63	0.28	2.58
Min	−1.56	−27.82	8.80	0.10	0.72
SD	1.4	2.0	1.5	0.04	0.46
AM – 2022					
Mean	2.59	−22.46	13.22	0.14	1.44
Max	7.27	−18.51	15.82	0.20	2.68
Min	−2.06	−27.02	10.94	0.06	0.56
SD	1.5	2.24	1.3	0.03	0.39
TM – 2023					
Mean	4.02	−23.23	10.8	0.17	1.29
Max	7.88	−19.15	18.0	0.33	2.46
Min	−6.40	−28.32	6.17	0.09	0.69
SD	2.5	2.17	2.18	0.04	0.32
AM – 2023					
Mean	1.24	−22.02	11.82	0.18	1.47
Max	9.55	−17.52	16.05	0.30	2.66
Min	−5.36	−27.68	7.18	0.07	0.70
SD	2.35	2.48	2.1	0.04	0.40

(Fig. 9b). In 2023 however, these differences were less pronounced (mean C:N of 11.8 in AM and 10.8 in TM). The relatively enriched $\delta^{13}C$ signatures and lower C:N ratios observed in TM soils may reflect enhanced turnover and mineralization of organic matter associated with greater C4 plant inputs, consistent with previous studies reporting faster decomposition dynamics of C4-derived residues under tropical conditions (Wynn et al 2020; Amundson et al. 2003). Typically, soils with a moderate C:N ratio (between 10–20) are considered optimal for microbial activity and balanced nutrient cycling (Haney et al. 2012).

Correlation analysis revealed consistent relationships among soil properties across agroecologies, particularly a positive association between %C and %N, indicating a close coupling between carbon and nitrogen dynamics. In contrast, isotopic relationships differed between systems, with TM soils showing a positive association between $\delta^{15}N$ and $\delta^{13}C$, unlike the weak negative correlations observed in AM soils and the overall dataset, suggesting distinct carbon and nitrogen processing pathways between agroecologies. Detailed correlation heatmaps are provided in Annex II.

Weed isotops

The carbon isotopic analysis reveals a clear separation between C_3 and C_4 photosynthetic pathways, as evidenced by two well defined clusters in the $\delta^{13}C$ values (Fig. 10a). Values of$\delta^{13}C$ range from approximately −35‰ to −10‰, with C_3 species characterized by

Table 4 The ANOVA agroecology, year, and their interaction as explanatory factors

Variable	Factor	sum_sq	df	F	p_value
% N	"Aroecology"	0.000	1	0.096	0.7573
% N	"Year"	0.019	1	9.822	0.0019**
% N	"Aroecology" + "Year"	0.000	1	0.031	0.8613
% C	"Aroecology"	1.860	1	13.262	0.0003***
% C	"Year"	0.032	1	0.228	0.6332
% C	"Aroecology" + "Year"	0.000	1	0.002	0.9668
C/N	"Aroecology"	138.858	1	32.624	0.0000***
C/N	"Year"	18.526	1	4.353	0.0378*
C/N	"Aroecology" + "Year"	0.029	1	0.007	0.9337
d15N ‰	"Aroecology"	726.061	1	137.541	0.0000***
d15N ‰	"Year"	50.935	1	9.649	0.0021**
d15N ‰	"Aroecology" + "Year"	8.272	1	1.567	0.2116
d13C ‰	"Aroecology"	48.878	1	9.243	0.0026**
d13C ‰	"Year"	0.247	1	0.047	0.8291
d13C ‰	"Aroecology" + "Year"	6.013	1	1.137	0.2871

n AM = 151; n TM = 145.

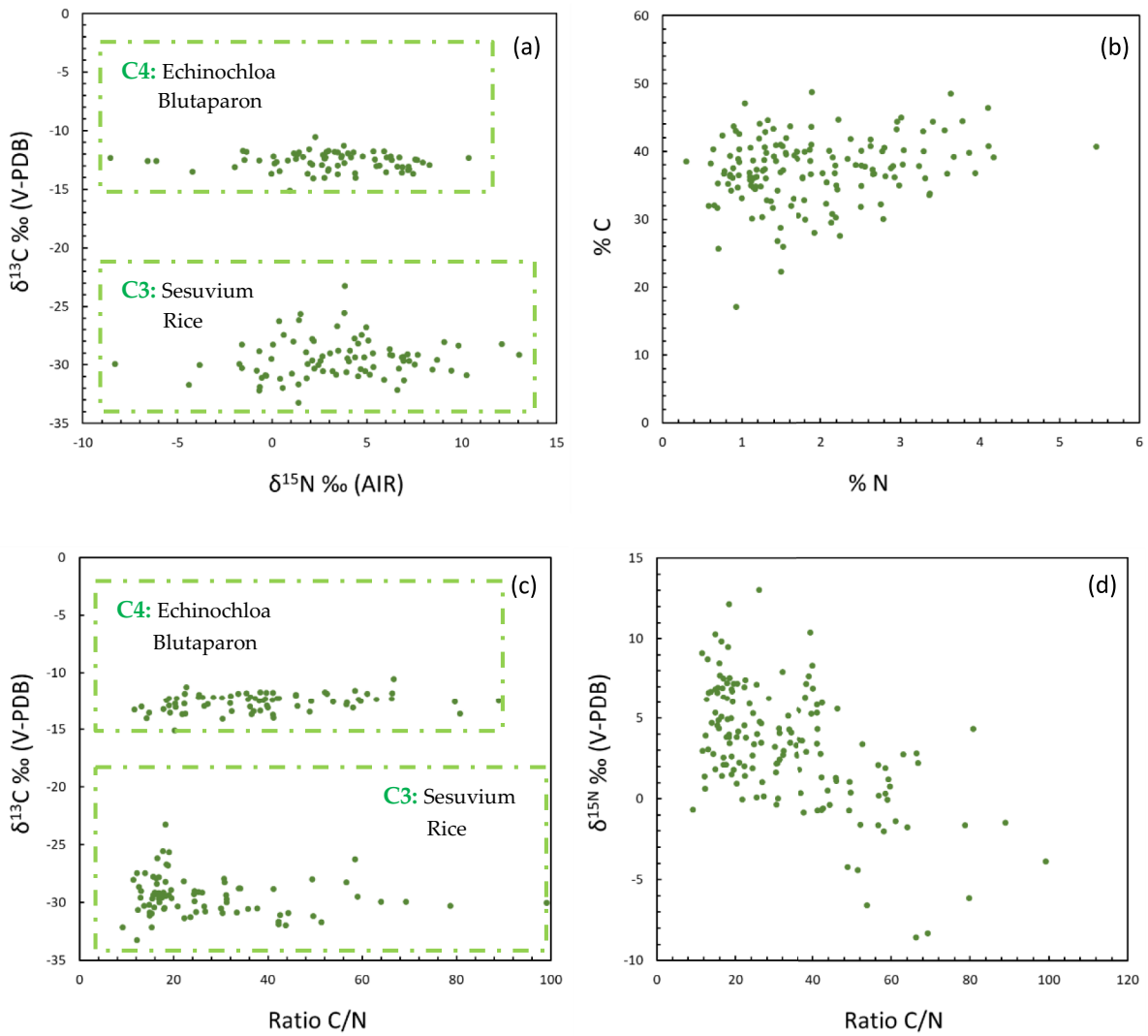

Fig. 10 (**a**) Isotopic $\delta^{13}C$ vs $\delta^{15}N$; (**b**) C to N in %; (**c**) isotopic $\delta^{13}C$ and C/N ration and (**d**); isotopic $\delta^{15}N$ and C/N ration for all sampled species (n = 154) from sampling 2022 and transect campaign July 2023

Table 5 One-way Anova and descriptive statistics for C3 versus C4 weed species, for the field campaign 2022–2023 (n = 154)

Variable	Mean C4	Max C4	Mean C4	SD C4	Mean C3	Max C3	Min C3	SD C3	F_ANOVA	p_value
% N	1.54	3.94	0.60	0.66	2.17	5.46	0.30	1.05	19.25	$2.13E^{-05}$***
% C	37.84	44.64	29.51	3.96	36.69	48.73	17.08	5.58	2.11	0.1483
C:N	38.37	88.97	11.70	17.29	27.86	99.18	9.20	16.90	14.52	0.0002**
d15N ‰	3.13	10.37	−8.54	3.66	3.45	13.04	−8.29	3.61	0.28	0.5962
d13C ‰	−12.88	−10.57	−15.14	1.98	−29.30	−23.28	−33.26	2.55	1958.66	$9.79E^{-89}$***

Table 6 Two-way Anova for plant variables with agroecology, year, plant type, and their interaction as explanatory factors

Variable	Factor	sum_sq	df	F	p_value
% N	"Aroecology"	4.229	1	2.899961	0.090
% N	"Year"	3.085	1	4.231768	0.041*
% N	"Plant Type"	11.609	1	15.91927	0.0001***
% N	"Aroecology" + "Plant Type"	3.531	1	2.421549	0.092
% C	"Aroecology"	114.863	1	2.433489	0.0912
% C	"Year"	9.713	1	0.411	0.522
% C	"Plant Type"	63.701	1	2.699	0.102
% C	"Aroecology" + "Plant Type"	15.33	1	0.324	0.723
C:N	"Aroecology"	694.006	1	1.279	0.281
C:N	"Year"	1243.002	1	4.583	0.033*
C:N	"Plant Type"	3089.924	1	11.394	0.0009***
C:N	"Aroecology" + "Plant Type"	1182.378	1	2.180	0.116
d15N ‰	"Aroecology"	58.263	1	2.431	0.091
d15N ‰	"Year"	19.943	1	1.664	0.199
d15N ‰	"Plant Type"	4,265	1	0,355	0.551
d15N ‰	"Aroecology" + "Plant Type"	172.692	1	7.205	0.0010**
d13C ‰	"Aroecology"	20.515	1	2.027	0.135
d13C ‰	"Year"	2.475	1	0.489	0.485
d13C ‰	"Plant Type"	9846.569	1	1946.118	$4.75E\text{-}^{87***}$
d13C ‰	"Aroecology" + "Plant Type"	29.754	1	2.940	0.055

more negative $\delta^{13}C$ values (mean: −29.3‰, Table 5) and C_4 species by less negative $\delta^{13}C$ values (mean: −12.8‰) consist with the established literature (Bender 1971; Smith and Epstein 1971) (Fig. 10c, Table 5). This separation is highly significant, as confirmed by the one-way ANOVA ($F = 1958.66$, $p < 0.001$).

In contrast, the $\delta^{15}N$ values show a broad and overlapping range for both groups (C_4: −8.54‰ to 10.37‰; C_3: −8.29‰ to 13.04‰), with similar mean values (C4: 3.52‰, C3:3.05‰). The ANOVA indicates no significant difference between groups ($F = 0.28$, $p = 0.596$), suggesting comparable nitrogen sources or cycling processes.

Elementary composition reveals clear differences. Nitrogen content (%N) is significantly higher in C_3 plants (mean: 2.17%) than in C_4 (mean: 1.54%) as supported by the Anova results ($F = 19.25$, $p < 0.001$). Conversely, carbon content (% C) does not differ between groups (C_3 mean: 36.69% and C_4 mean: 37.84%, $F = 2.11$, $p = 0.148$), although C_3 plants show greater variability (ranging from 17.08% to 48.73%).

The C:N is significantly higher in C_4 plants (mean: 38.71) compared to C_3 plants (mean: 27.37) (Fig. 10c,d; Table 6), indicating lower nitrogen content and potentially more lignified or structurally complex tissues in C_4 biomass. This pattern is consistent with the observed lower %N in C4 species.

Overall, these results highlight distinct physiological and biochemical traits between C_3 and C_4 weeds. While $\delta^{13}C$ effectively separates the two functional groups, differences in %N and C:N ratio further suggest contrasting nutrient use strategies and potential implications for soil organic matter quality and nutrient cycling in the agroecosystem. However, $\delta^{15}N$ values do not differ significantly, indicating similar nitrogen dynamics across both plant types.

The ANOVA analysis (Table 6) indicates that plant isotopic and elemental composition is primarily controlled by plant type, with additional but weaker effects of year and limited influence of agroecology. Plant type had a highly significant effect on $\delta^{13}C$, %N, and C:N ratio, confirming strong physiological differences between C_3 and C_4 species. Year also showed significant effects on %N and C:N ratio, suggesting some interannual variability in nutrient dynamics. In contrast, agroecology did not exert a significant effect on any variable when considered alone. For $\delta^{15}N$, no main factor was significant; however, a significant interaction between agroecology and plant type was

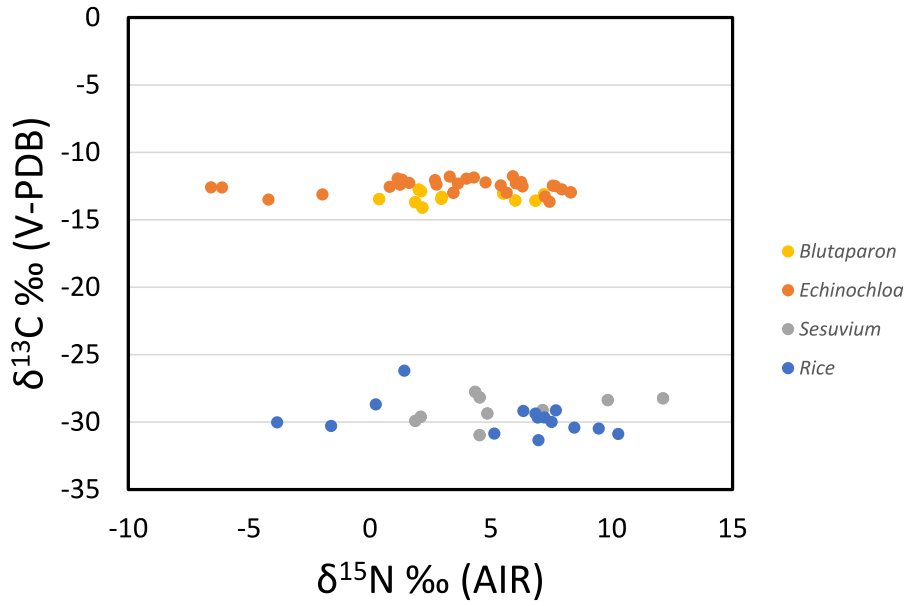

Fig. 11 Main weed species $\delta^{13}C$ to $\delta^{15}N$ isotopic distribution (n = 85)

detected, indicating that nitrogen isotopic composition is influenced by the combined effect of environmental conditions and plant functional group. Overall, these results highlight plant type as the dominant driver, with secondary temporal effects and context-dependent interactions for nitrogen dynamics.

Plant correlation analysis revealed consistent relationships between elemental composition and isotopic variables across agroecologies. In both TM and AM systems, %N was strongly negatively correlated with the C:N ratio, while $\delta^{15}N$ showed a moderate positive association with %N, particularly in AM plants. In contrast, $\delta^{13}C$ relationships differed between agroecologies, with TM plants exhibiting stronger associations with C:N ratio and %N, suggesting clearer physiological differentiation between C_3 and C_4 functional groups under TM conditions. Detailed correlation heatmaps are provided in Fig. 3, Annex II.

The main identified weed species also displayed distinct isotopic signatures (Fig. 11). *Echinochloa colona* and *Blutaparon vermiculare* display consistent $\delta^{15}N$ values while *Sesuvium portulacastrum* exhibited more depleted $\delta^{13}C$ values and grater $\delta^{15}N$ variability, indicating differences in nitrogen uptake and environmental adaptation. However, *Sesuvium portulacastrum* cannot be considered C3 plants sensu stricto, although they obtain CO_2 principally via the C3 pathway (Winter et al. 2019). Consequently, this homogeneous and very negative values indicate that water stress is not present, clearly indicating its C_3 photosynthetic pathway. Rice, used as a reference crop, also plots within the C_3 range with $\delta^{13}C$ values around −29‰ to −31‰ and $\delta^{15}N$ values from approximately 2‰ to 11‰, overlapping partially with *Sesuvium.*

Consistent with these observations, soil organic matter from both TM and AM sites show a dominant C3 isotopic signature (Fig. 9), suggesting a strong contribution from C_3 plant residues, most likely from the incorporation of rice straw and Sesovium (especially in the TMs) into the soil. As rice is a C_3 crop, its post-harvest biomass inputs—such as root residues and surface-applied straw—likely represent a major source of organic carbon, influencing the $\delta^{13}C$ signature of the soil.

Interaction soil, weeds and water

The principal component analysis (PCA) biplot revealed a clear separation between soil and plant samples across the two agroecological systems (AM and TM), as well as between plant functional types (C3 and C4). This differentiation is primarily structured along the first two principal components, with PC1 explaining 44.8% and PC2 accounting for 26.7% of the total variance (Fig. 12A). The separation between soil and plant clusters is mainly driven by PC2, where the strongest loadings correspond to

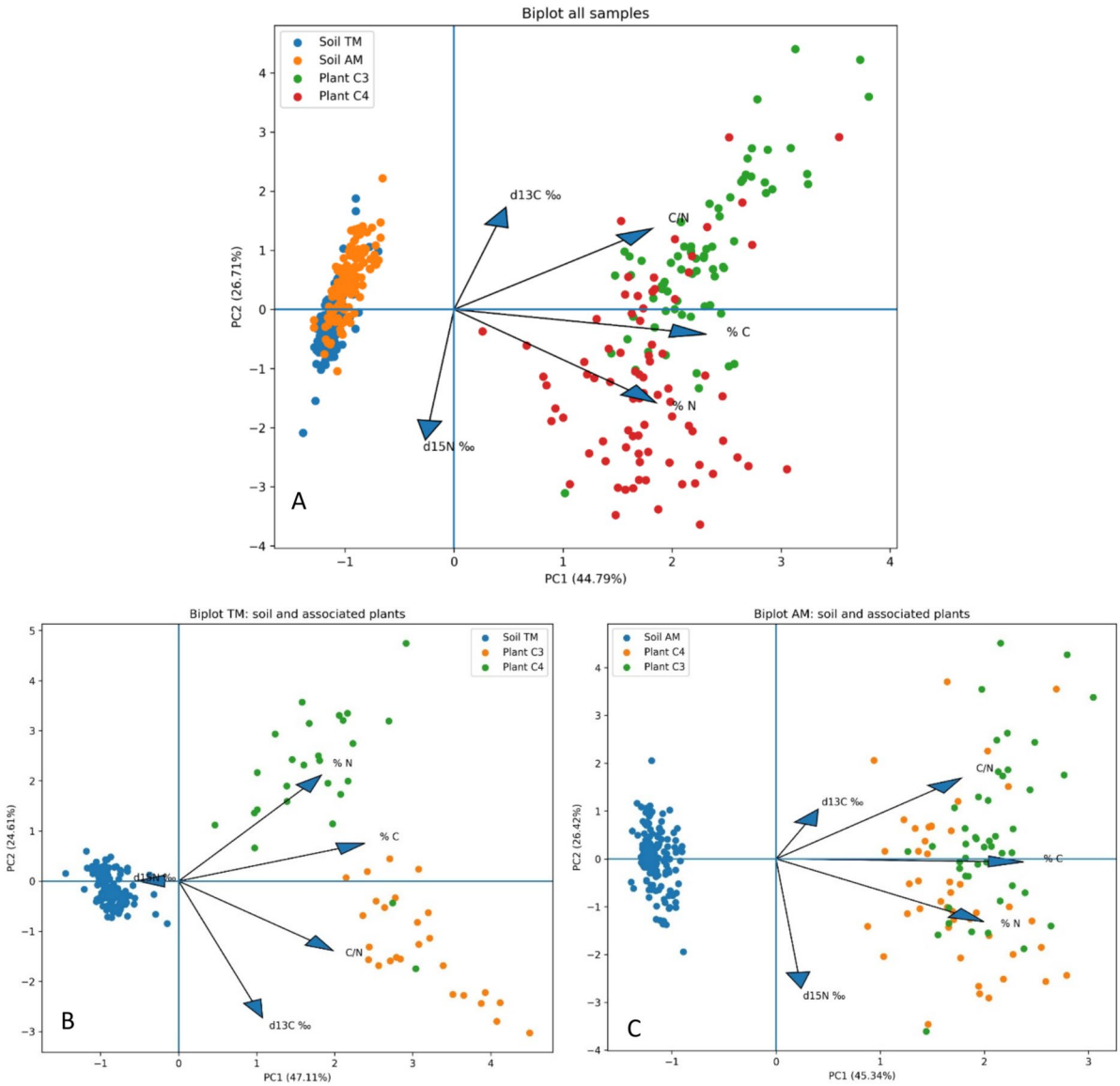

Fig. 12 Principal component analysis (PCA) biplots illustrating the relationship between soil and plant variables; A. Combined PCA for both agroecological systems (AM and TM); B. PCA biplot for soils and associated plant species in the TM agroecology; C. PCA biplot for soils and associated plant species in the AM agroecology

$\delta^{15}N$ (−0.63) and $\delta^{13}C$ (0.49), with a smaller contribution from the C:N ratio (0.39). This indicates that isotopic signatures, particularly $\delta^{15}N$ and $\delta^{13}C$, are the most robust variables distinguishing the origin and processing of organic matter in soils versus plants. In contrast, PC1 is largely associated with bulk elemental composition, with high positive loadings for %C (0.65), %N (0.52), and C:N ratio (0.51). This suggests that carbon and nitrogen pools are closely linked across both soils and plants, reflecting shared biogeochemical controls and nutrient cycling processes within the system. The orientation of vectors further suggests that plant samples are more strongly associated with higher %C and %N variability, while soil samples are more influenced by isotopic composition, reinforcing the role of isotopic

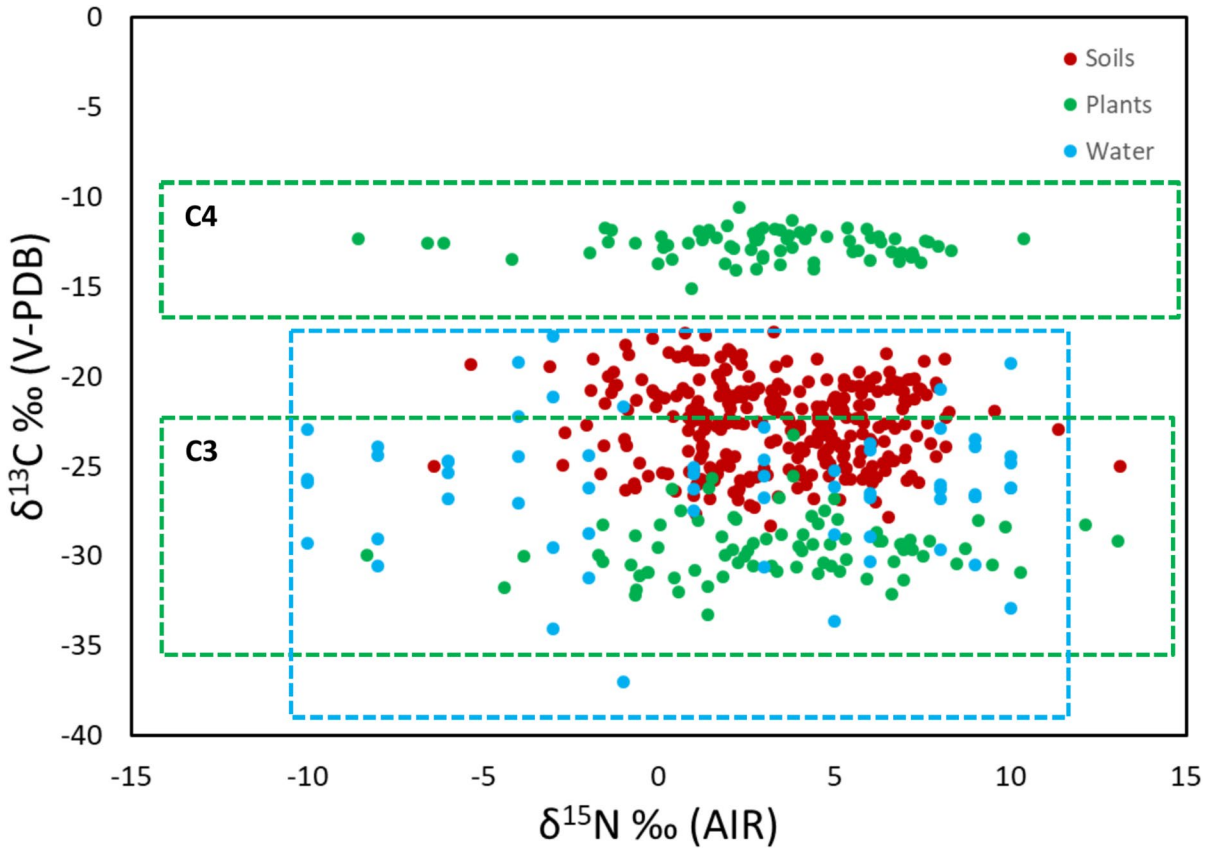

Fig. 13 Nitrogen $\delta^{15}N$ and Carbon $\delta^{13}C$ isotopic signatures in soils (red), plants (green), and POM- (blue), including overall samples for 2022 and 2023. ***Note:*** The $\delta^{15}N$ values for water samples are approximated for visualization purposes, as all measured values were recorded as 0‰. This adjustment was made to allow for consistent and comparative representation across all sample types

tracers in distinguishing organic matter sources and transformations.

When the PCA was performed separately for each agroecological system, distinct patterns emerged between TM and AM. In the TM agroecology (Fig. 12B), plant functional types (C3 and C4) showed a clearer separation along PC2, mainly associated with $\delta^{13}C$, %N, and C:N ratio, indicating stronger isotopic differentiation and nutrient-use contrasts between vegetation groups. In contrast, the AM agroecology (Fig. 12C) displayed greater overlap between C3 and C4 plants, with %C and C:N ratio contributing more strongly to sample distribution, suggesting a more mixed organic matter signature and less distinct functional separation.

Furthermore, isotopic linkage between soil, plant and water samples from AM and TM agroecological systems revealed that soil isotopic signatures fall within the characteristic carbon (C) and nitrogen (N) isotopic ranges of C3 and C4 plants (Fig. 13). However, the soils fall mostly within the C3 range, suggesting dominant organic matter input from C3 vegetation, which is in agreement with anthropogenic induction (introduction of crops) and use of irrigation, factors (even in this agroecologies the irrigation term means only rainfed water management system) that have both favored C3 photosynthesis pathways.

Most water samples show $\delta^{13}C$ values closely aligned with those of C3 plant-derived organic matter, suggesting a substantial contribution of particulate organic matter (POM) from surrounding terrestrial vegetation. An additional contribution likely originates from in situ primary production within the aquatic system (e.g., algae and phytoplankton).

However, plant decomposition alone may not fully account for the composition of organic matter in the soil–water interface. Notably, the $\delta^{13}C$ values of water samples display a wide range, indicative of complex hydrological mixing and temporal variability. For instance, water collected during the 2022 wet season exhibited more negative $\delta^{13}C$ values (mean: −27‰), while samples from the 2025 dry season were comparatively less negative (mean: −23‰). Although C4 plants are abundant in the active mangrove (AM) fields and were expected to significantly influence the isotopic signals, their impact appears diminished. This can be explained by findings from Wynn and Bird (2007), which show that C4-derived soil organic carbon (SOC) decomposes more than twice as fast as C3-derived SOC in mixed C3/C4 systems. Such rapid turnover likely contributes to the more dynamic and transient presence of C4 signals in both soil and water. Overall, the isotopic composition of water reflects an integration of plant-derived organic inputs and microbial activity, emphasizing the interconnectedness of hydrology and nutrient cycling within the ecosystem.

Discussion

Extensive and continuous fieldwork with the local farmers between 2021 and 2023 revealed marked spatial variations in soil processes, nutrient dynamics, and vegetation composition across the main agroecological systems (Merkohasanaj et al. 2025a,b; Garbanzo et al. 2024, 2025). These findings underline the strong ecological heterogeneity of the mangrove swamp rice production (MSRP) system in Guinea Bissau and reinforce the need for localized and adaptive management strategies (Temudo and Santos 2017).

One of the central observations relates to the influence of soil physical properties particularly soil texture and water retention capacity, on decomposition rates, microbial biomass and nutrient mineralization. Fine-textured soils generally contain higher proportions of C and N in microbial biomass but exhibit lower mineralization rates per unit of biomass compared to coarse-textured soils (Hassink 1994). Recent studies further demonstrate that water availability and soil properties act as major environmental filters shaping microbial communities in agroecosystems (Onoszko et al. 2025). These findings highlight the complex interactions between soil properties, hydrological regime, organic inputs, and microbial processes in controlling nutrient cycling and organic matter turnover.

In natural low-latitude environments, forested areas are typically dominated by plants using the C_3 photosynthetic pathway, while open grasslands are often characterized by C_4 species, such as Poaceae grasses, resulting in more enriched $\delta^{13}C$ values in both vegetation and soil organic matter (Bender 1971; Cerling 1984; Peterson and Fry 1987). However, the mangrove rice production systems studied here in Guinea-Bissau present a markedly different pattern due to long-term anthropogenic influence. The systematic introduction of rice—a C_3 crop—together with long-term water management practices, has progressively shifted the isotopic composition of soils and waters toward more depleted $\delta^{13}C$ values, characteristic of C_3 photosynthesis (Table 3, Figs. 9 and 13).

This dominant C_3 isotopic signal in soil organic matter (−17.52‰ to −28.32‰ *vs* V-PDB) persist even in fields where C_4 grasses such as *Echinochloa colona* remain visually abundant.

This pattern likely reflected both the contribution of C_3—derived residues (e.g., rice straw, *Sesuvium* spp.) and the faster turnover of C_4-derived organic matter, as previously reported in tropical mixed C_3/C_4 systems (Wynn and Bird 2007). The relatively enriched $\delta^{13}C$ signatures and lower C ratios observed in TM soils may therefore indicate enhanced organic matter turnover associated with greater C_4-derived inputs. However, these interpretations remain indirect proxies rather than direct measurements of decomposition rates, which would require approaches such as biomass decomposition experiments, soil respiration measurements, or carbon mineralization assays.

Isotopic analysis of particulate organic matter (POM) in water samples further supports the predominance of C_3-derived organic matter. Most waterborne organic matter shows $\delta^{13}C$ values consistent with terrestrial C_3 vegetation, indicating a dominant contribution from plant residues rather than aquatic primary producers. Seasonal differences in $\delta^{13}C$ values suggest that temporal dynamics -such as rainfall, flooding, and decomposition stage influence organic matter composition at the soil–water interface. For instance, more negative $\delta^{13}C$ values were observed during the wet season (−27‰), likely due to increased input of fresh C_3 detritus from rice and weeds.

From an ecological perspective, these findings demonstrate the effectiveness of stable isotope analysis as a tool to trace changes in land use and water management in traditional agroecosystems. The predominance of C_3-derived organic matter reflects the cumulative effect of anthropogenic pressure and adaptive agricultural practices, including crop selection and water regulation. These signals are especially valuable for understanding nutrient cycling and soil fertility dynamics in low –input systems where external amendments are largely absent.

The isotopic composition of nitrogen ($\delta^{15}N$) in soils and weeds across the AM and TM agro-ecologies revealed meaningful differences in nitrogen cycling and availability (Table 3, Fig. 13). TM soils displayed higher and broader $\delta^{15}N$ values compared to AM soils, suggesting greater nitrogen mineralization and microbial activity in TM fields, potentially associated with improved aeration and drainage conditions (Amundson et al. 2003; Hassink 1994).

In contrast, the lower and narrower $\delta^{15}N$ values observed in AM soils are consistent with more conservative nitrogen cycling under wetter and less oxygenated conditions (Högberg 1997). Plant $\delta^{15}N$ values also varied among weed species and functional groups, although no significant differences were detected between C_3 and C_4 plants, suggesting broadly similar nitrogen cycling pathways across vegetation types.

The broad $\delta^{15}N$ range in both soils and vegetation (from approx. $-6‰$ to $+13‰$) further supports the presence of mixed nitrogen sources and complex biogeochemical processes, influenced by soil type, water management, and vegetation dynamics. These values also reflect low external N input, aligning with the traditional, low-input character of mangrove rice systems (Amundson et al. 2003; Craine et al. 2009).

Moreover, the integration of drone-based weed monitoring with isotopic data offers promising avenues for mapping bio-indicator species and predicting zones of differential nutrient turnover and evaluating spatial variability across agroecological systems. The context-specific nature of spontaneous vegetation as a bio-indicator was evidenced by the clear separation between TM and AM agroecologies, driven by differences in water availability, salinity, slope, and related soil conditions. For example, the predominance of halophytic species such as *Blutaparon vermiculare* in TM plots contrasted with the greater abundance of *Echinochloa colona* and more heterogeneous vegetation assemblages in intermediate and upper AM plots, reflecting localized hydrological and edaphic gradients. This variability was observed both within individual transects and between village, where the Cafine transects supported different plant communities compared to those observed in Malafu. Together thisfindings highlight the potential of UAV-based approaches for future large-scale ecological assessment and monitoring.

Future research should investigate the influence of other potential contributors—such as riverine input, precipitation, and surface runoff—on the isotopic composition of soils and aquatic organic matter. In parallel, seasonal monitoring could clarify whether isotopic signals from C_4 species persist or fluctuate across varying hydrological and management conditions.

Advancing weed identification and functional classification will depend not only on strengthened taxonomic frameworks but also on the systematic integration of farmers' locally grounded knowledge. Through long-term social interactions, shared practices, and experiential learning, farmers develop a nuanced understanding of weed behavior, competitive relationships, and context-specific management strategies. Recent studies by Leunda et al. (2024) highlight how such socially embedded knowledge systems play a central role in shaping adaptive agricultural practices and decision-making processes. When combined with scientific evidence, this co-produced knowledge can help bridge critical gaps, enhance ecological relevance, and foster more effective, community-driven innovation in agroecosystem management.

Conclusions

This study highlights the strong influence of long-term management and hydrological regulation on the isotopic composition of soils, vegetation and waters in mangrove rice systems of Guinea-Bissau. The predominance of $\delta^{13}C$ values characteristic of C_3 plants in soil and particulate organic matter reflects the cumulative effects of rice cultivation and irrigation practices, which have favored C_3 vegetation over naturally occurring C_4 grasses. These isotopic patterns offer valuable insights into organic matter turnover, nutrient cycling, and the ecological legacy of low-input rice production systems.

Differences in $\delta^{15}N$signatures between TM and AM agroecologies further indicate contrasting nitrogen

cycling dynamics, with TM soils showing evidence of enhanced mineralization and microbial processing, while AM systems reflect more conservative nitrogen turnover under wetter conditions. Together, these findings highlight the importance of hydrology, vegetation composition, and soil properties in structuring biogeochemical processes across mangrove rice agroecosystems.

The integration of stable isotope analysis with UAV-based vegetation monitoring proved effective for identifying spatial patterns in weed communities, vegetation density, and agroecological variability. UAV classification showed high accuracy in distinguishing dominant plant communities and revealed clear differences in vegetation structure between TM and AM systems. These combined approaches offer promising opportunities for ecological monitoring, bioindicator identification, and site-specific management in traditional low-input farming systems.

Building upon the results of this study, several avenues for future research and development can be proposed to improve both scientific understanding and local management practices in mangrove rice systems:

- Characterize the fertility potential of individual rice plots using bioindicators by field experimentation of high fertile plots versus low fertile plots. This would enable more targeted soil management strategies based on specific vegetation dynamics.
- Develop and test decision-support tools for farmers, aimed at optimizing the management of both beneficial and competitive weed species. Integrating local ecological knowledge with modern monitoring techniques may empower smallholders to enhance crop productivity and resilience.
- Refine isotopic techniques and drone-based classification methods to enhance the spatial resolution and accuracy will allow better tracking of nutrient cycling processes over time and space.

These objectives are key to reinforcing sustainable agricultural practices in low-input systems, particularly under climate and socio-economic pressures. Advancing these lines of research will contribute to both agroecological conservation and the food security of coastal communities in Guinea-Bissau. Therefore, fully integrating these methods into a coherent, farmer-centered strategy remains essential to develop practical, scalable solutions rooted in both scientific evidence and local knowledge.

Acknowledgements A grateful acknowledge for the support goes to the Soil and Water Laboratory in Bissau, the Soil Laboratory at the School of Agriculture (ISA), University of Lisbon, and the Soil and Agricultural Chemistry Laboratory at the University of Granada (UGR). The authors extend especial gratitude to Merlin Leunda-Martiarena, Juvinal Silva Santos, Adinane Jalo, Paulina Na-Bitchom, Eduino Mendes da Costa, Adriano Barbosa and Alqueia Intchama for their unconditional support, meticulous sample collection and laboratory analyses, and hard field work in the study sites of Guinea-Bissau.

Author's contributions Conceptualization, M.M., A.A., and A.D.; Data curation, M.M. and J.C.; Formal analysis, M.M.; Investigation, M.M and A.D.; Methodology, M.M., A.A., N.C., C.Q., L.G., and A.D.; Software, M.M. and J.C; Supervision, A.A., N.C., C.Q., L.G., and A.D.; Validation, M.M., J.C., A.A. and A.D.; Visualization, M.M. and J.C.; Project administration, Resources and Funding acquisition, M.T.; Writing—original draft, M.M.; Writing—review & editing, N.C., A.A., C.C., L.G., A.D. and M.T.

Funding Funding for open access publishing: Universidad de Granada/CBUA. This work was done with the financial assistance of the European Union, DeSIRA project 'Mangroves, mangrove rice and mangrove people: sustainably improving rice production, ecosystems and livelihoods' (Grant Contract FOOD/2019/412–700).

Data Availability The image classification data and associated code used for weed detection analyses are publicly available at https://github.com/Emmanuel461/P4M_Weed_detecction. Additional datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflicts of interest The authors declare no conflicts of interest.

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