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Research article

From field to farm: Valorization of tobacco cv. *Solaris* biomass as non-conventional silage

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ABSTRACT

This study presents an integrated field-to-farm approach for the valorization of *Nicotiana tabacum* L. cv. *Solaris* as a non-conventional silage, cultivated in Central-South Italy. The experimental design integrated agronomic management of whole-plant biomass, lactic acid bacterial inoculant selection and validation, and evaluation of nutritional, chemical, and microbiological traits of the resulting silage across two consecutive seasons, based on 2 ha of cultivation per year. The ensiled cv. *Solaris* biomass (SiloSolaris) exhibited a balanced nutritional profile, with crude protein contents of 16.8–17.0 g 100 g^{-1} dry matter (DM), structural carbohydrate levels suitable for ruminal fermentation (NDF 45–48 g 100 g^{-1} DM), and consistently low alkaloid concentrations (0.11–0.46 g nicotine 100 g^{-1} DM), supporting its suitability for ruminant feeding. A targeted biotechnological strategy based on *Lactiplantibacillus plantarum* LPUM1 was applied as a silage inoculant, either alone or delivered through cheese whey as a functional carrier. Inoculated silages via whey showed improved fermentation kinetics, enhanced dominance of lactic acid bacteria, and reduced undesirable microorganisms, including eumycetes and *Clostridium* spp. Overall, cv. *Solaris* can be integrated into ruminant diet when agronomic practices, ensiling conditions, and microbial management are coherently aligned. Beyond the specific crop investigated, the proposed field-to-farm approach provides a transferable model for stabilizing and upgrading non-food crops and agro-industrial co-products into safe, stable and nutritionally functional silages, contributing to circular resource use in livestock production systems.

1. Introduction

Population growth and increasing demand for arable lands are driving the agricultural sector to increase productivity and efficiency, with the aim of creating an efficient value chain that satisfies target markets, reducing environmental impact and protecting animal welfare (Khanal, 2024). Identification, characterization, and use of alternative feed sources, locally available and less

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competitive with human food, will be essential for the development of sustainable livestock production (Chisoro et al., 2023; Khanal, 2024). Co-products from agro-industrial plants represent a promising source of dietary ingredients for animals either directly or after processing, rich in energy, protein, and other nutrients, as well as of beneficial bioactive compounds (Skoufos et al., 2023).

Tobacco (*Nicotiana tabacum* L.) is considered one of the world's most abundant industrial non-food crops, and represents the most profitable agricultural activity in many regions of the world (Hiscock et al., 2023; Mavroeidis et al., 2024; Liu et al., 2025). China is the largest producer of tobacco worldwide, followed by Brazil and USA, while Italy is the largest producer within the European Union (MASAF, 2026). Due to the smoking tobacco crisis, the tobacco sector is trying to diversify focusing on the reuse of co-products in a circular economy perspective (Grisan et al., 2016). The co-products derived from tobacco cultivation and manufacture, *i.e.*, leaves, stems, and processing residues, could be properly valorized by promoting their reuse, thus exerting a favorable impact on environmental sustainability (An et al., 2025).

In this context, the case of *Nicotiana tabacum* cv. *Solaris* (International Patent PCT/IB/2007/053412; Fogher, 2008), an innovative no-genetically modified organisms tobacco variety, represents a shift towards more sustainable and multifunctional tobacco culture. Unlike traditional smoking tobacco, this cultivar has been selected as an energy crop for oilseeds production (Fatica et al., 2025). In addition to biojet fuel, co-products derived from cv. *Solaris* cultivation, including plant biomass and seed cake obtained after oil extraction, can be successfully used as animal feed (Rossi et al., 2013; Fatica et al., 2021, 2025), enhancing the sustainability of its supply chain.

Tobacco cv. *Solaris* is characterized by limited leaf growth, prominent apical flower development, and strong vegetative capacity, which allow regrowth after flower harvest for seed production (Grisan et al., 2016; Fatica et al., 2019, 2021, 2025). In addition, it shows lower levels of total alkaloids expressed as nicotine (0.30–1.35 g/100 g dry matter, DM), compared to conventional smoking tobacco (Fatica et al., 2025). In this regard, the valorization of agro-industrial co-products as alternative feed resources represents a strategic approach to enhance the sustainability and resilience of livestock systems. The inclusion of co-products in animal diets may reduce feeding costs, which account for approximately 70% of total livestock production costs (Rohela et al., 2025). When properly evaluated in terms of cultivation conditions, storage practices, and chemical composition, selected co-products can partially replace conventional feed sources without compromising animal performance, while improving resource-use efficiency and overall system sustainability.

Ensiling, the most important method for forage preservation worldwide, is performed in anaerobic environment, which limits the growth of aerobic spoilage microorganisms, *i.e.*, molds, yeasts, bacteria, and allows the fermentation of sugars to organic acids, lactic acid in particular (Muck et al., 2020). As previously reported (Fatica et al., 2021), cv. *Solaris* plants are suitable for the ensiling process and could represent a valuable alternative forage resource for ruminants. However, the integration of this innovative co-product into livestock diets requires a comprehensive understanding of tobacco cultivation techniques and potential microbiological and pathological concerns associated with tobacco silage utilization. Nevertheless, experimental data are still required to better define its agronomic and fermentative performance under different cultivation conditions and to support its potential use as an alternative silage resource.

This study aims to evaluate the potential of *Nicotiana tabacum* cv. *Solaris* whole plants as a non-conventional silage by assessing agronomic plant management, ensiling suitability, and nutritional, chemical, and microbiological characteristics of the ensiled biomass. Emphasis was given to plant growth performance, biomass yield, and optimization of the ensiling process. Chemical composition and main microbiological characteristics of the cv. *Solaris* silage were also evaluated.

2. Materials and methods

2.1. Study area characterization

The study was conducted in two experimental fields located in San Giorgio del Sannio municipality (Benevento province, IT), over two consecutive cultivations years (41.103°N 14.877°E, 2021/2022 as year I; 41.113°N 14.883°E, 2022/2023 as year II), on a total area of two hectares per year located at about 350 m of altitude. The sites were located in Central-Southern Italy, in an area characterized by strong agricultural vocation and suitable pedoclimatic conditions for tobacco cultivation (Fig. 1a–b). The experimental design included three key aspects: i) agronomic management of whole plant biomass harvested at optimal physiological maturity; ii) selection and functional validation of a lactic acid bacterial inoculant; iii) comparison of two consecutive cultivation seasons differing in specific technological variables.

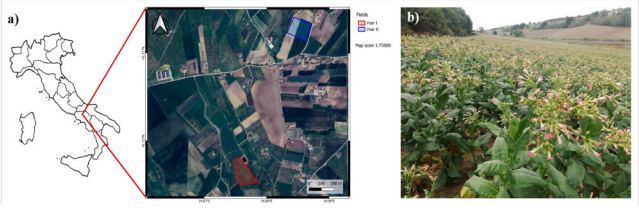

Fig. 1. Geographic location of the experimental fields over two-year period **(a)**; representative field view of *Nicotiana tabacum* cv. *Solaris* cultivation **(b)**.

Fields were characterized by medium depth, poor skeleton, loamy-clayey texture, medium organic matter content and poor drainage. Soil management practices, and fertilizer and insecticide treatments, were made according to the field characteristics and to the previous crop type, *i.e.*, winter cereal (*Triticum durum* Desf.), applying the same agronomic protocol during the two years of cultivation. Continuous cropping was avoided at the cultivation sites, since it can lead to soil problems such as reduced soil depth, structural degradation, and nutrient imbalances, which negatively affect tobacco growth and development, reducing both yield and quality (Yan et al., 2024).

Climatic data, including both average monthly temperature (°C) and monthly rainfall (mm) for the two years of cultivation were collected from the official archive of Regione Campania (2024). Data were analyzed and represented in a thermo-pluviometric diagram (Fig. 2) according to Bagnouls and Gaussen (1957). Briefly, in year I the total annual precipitation was 971 mm, with November as the rainiest month (239.4 mm) and August as the driest (5.2 mm). The higher temperature was recorded in August (25.4 °C), while January was the coldest month (6.4 °C). In year II, total annual precipitation was 1021 mm, with November again as the rainiest month (186.6 mm). July was the driest (23.8 mm) and also the hottest (25.4 °C), while January was the coldest (6.3 °C).

2.2. Agronomic management

After medium-deep plowing (40 cm of depth) followed by minimum tillage (20 cm of depth), transplantation of *Nicotiana tabacum* cv. *Solaris* plants occurred during the second decade of May, respectively two weeks after the distribution of ternary fertilizer (500 kg ha^{-1}) with 12:12:17 N:P:K ratio, and one week after the weed chemical control carried out using pre-emergence weeding through a pendimethalin based product. Tobacco cv. *Solaris* plants were mechanically transplanted into the selected fields following a 0.90 m (between rows) × 0.31 m (along the row) pattern in year I, and 0.90 m × 0.35 m pattern in year II, resulting in 36,000 plants per hectare and 32,000 plants per hectare, respectively. In year II, the slight difference in plant densities was adopted to explore crop response under different conditions, and to facilitate specific field operations, including the harvesting of floral apexes for seed production. Mechanical weeding and additional nitrogen fertilization (overall 500 kg ha^{-1} of ammonium nitrate, 34% of N) were carried out twice during each cultivation season, at fourth and tenth weeks after the tobacco' transplantation, respectively. Three pest control treatments against tobacco cutworm (*Spodoptera litura* Fabricius) were carried out during each cultivation season, immediately after transplanting, four weeks after transplanting, and after the floral apexes harvest. Treatments were performed using pyrethroids and *Bacillus thuringiensis* -based products, applied at rates consistent with the label recommendations of the commercial products used. In both years of tobacco cultivation, the deficiency period of plant protection products used was respected, and the harvest did not take place earlier than 60 days after the last treatment.

Fields were irrigated by a sprinkler system four times during year I, and once during year II, providing around 20 mm of water at each irrigation. Irrigation timing and volume were defined by tobacco cv. *Solaris* water requirements and water availability, also according to climatic conditions and seasonal precipitations. Ninety days after the transplanting, tobacco cv. *Solaris* floral apexes were mechanically harvested, while green biomass was harvested after further 60–70 days, as described below.

To evaluate the morphological characteristics of tobacco cv. *Solaris* plants, one week before the green biomass collection in each cultivation year, whole plants (n = 20) were collected. Briefly, considering one hectare as a reference area, sampling was carried out

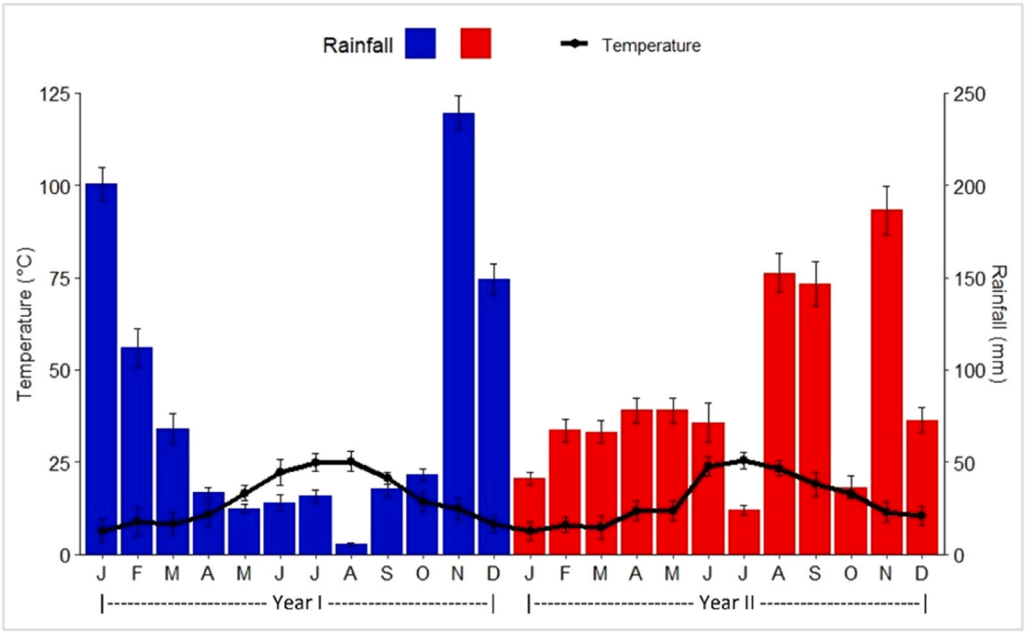

Fig. 2. Thermo-pluviometric diagram for the year I (on left) and year II (on right).

along the diagonal transect of the field. Five plants per hectare per year were sampled at regular intervals (28 m) along the diagonal transect, used as a representative sampling line across the experimental area.

Plant height was measured from the plant's collar to the floral apex. Leaves number was registered and leaf area index (LAI) was measured using LI-3100C area meter (LI-COR, Lincoln, USA). According to official methods (AOAC, 2000), moisture content was determined by drying leaves in a forced-air oven at 60 °C for 72 h.

2.3. Tobacco cv. Solaris biomass ensiling process

In fall, at the end of each cultivation year, *Nicotiana tabacum* cv. *Solaris* plants were harvested at the waxy ripening stage, when the average moisture content reached approximately 75%, chopping with a corn shredder at about 4 cm of length, as previously reported (Fatica et al., 2021). Harvesting was performed at 152- and 158-days after transplantation during the first and the second year of cultivation, respectively. The total amount of chopped biomass collected was 19.5 t (year I) and 17.8 t (year II).

At this stage, the fermentability and microbiological stabilization of the ensiled cv. *Solaris* biomass were evaluated through a dual approach: i) plant-scale ensiling trials conducted under real-farm conditions; ii) laboratory-scale silo bag models designed to simulate on-farm storage conditions by periodically monitoring the anaerobic fermentation process. Both ensiled trials employed the same lactic acid bacterial (LAB) inoculant, with differences in its application mode. During the first year, the inoculant was suspended in water, whereas in the second year it was delivered in cheese whey to explore the potential effect of this carrier on microbial implantation and fermentation kinetics. Thermally sanitized (pasteurized) whey, used as a carrier for the starter strain, was obtained from cow's milk coagulated with rennet. It was characterized as follows: near-neutral acidity (pH 6.4–6.6), and lactose 4.5–5.0% (w/v), protein 1.16 (± 0.06)%, ash 0.55 (± 0.02)%, lactic acid 0.21 (± 0.03)%, moisture 93.3 (± 0.11)% contents.

In both years, the cv. *Solaris* biomass was ensiled on farm in a bunker silo (7 m × 3 m × 1.2 m), carved into the ground at a commercial dairy farm (41.219°N, 14.784°E), and appropriately dimensioned to ensure a homogeneous use of the feed-out face during the unloading phase, thereby limiting oxygen exposure. The bunker silo was mechanically compacted and sealed with PVC cover (Fatica et al., 2019), over which a layer of about 30 cm of soil was placed to ensure anaerobic storage. During the second year, a 15 cm layer of straw was added to the bottom of the bunker silo as a functional modification to improve leachate management. The silos remained closed for 150 days in year I and 116 days in year II, respectively. For the laboratory scale, samples of chopped green biomass (n = 24, approximately 0.2 kg of each) inoculated or not were collected in vacuum-packed polyethylene bags to enable analytical monitoring under standardized conditions. This two-level characterization, combining raw material traits and tailored ensiling conditions, was essential to generate a robust dataset linking agronomic performance, technological interventions, and fermentative outcomes in the subsequent microbiological and physicochemical analyses.

2.3.1. Starter culture selection

The lactic acid bacterium inoculated in the ensiling trials was *Lactiplantibacillus plantarum* LPUM1, a strain previously isolated from spontaneous vegetable fermentations and maintained in the culture collection of the Department of Agricultural, Environmental and Food Sciences (DiAAA) of University of Molise. This strain was selected because it combines the acidification capacity with a broad antimicrobial spectrum, thus acting both as a fermentative driver and a bioprotective agent during the storage of *Nicotiana tabacum* cv. *Solaris* biomass. The antifungal and anti-clostridial activities of *L. plantarum* LPUM1 were evaluated *in vitro*. Antifungal activity was determined by the agar well diffusion method against *Aspergillus flavus*, *Fusarium graminearum*, and *Penicillium roqueforti* (from DiAAA collection). Potato dextrose agar (PDA) plates were seeded with 10^6 conidia mL^{-1} , incubated at 28 °C for 72 h, and inhibition zones were expressed in mm. Anti-clostridial effects were assessed in anaerobic co-cultures with *Clostridium butyricum* and *C. tyrobutyricum* (10^4 CFU mL^{-1}) in Reinforced Clostridial Medium at 37 °C, over 48 h, inoculated either in monoculture (controls) and in co-culture with *L. plantarum* LPUM1 (10^7 CFU mL^{-1}). Viable colonies were counted for samples collected at 0, 8, 24, and 48 h on selective agar media.

2.3.2. Plant-scale and vacuum-pack ensiling trials

Chopped whole plants of *Nicotiana tabacum* cv. *Solaris* were inoculated with the strain *L. plantarum* LPUM1, that was applied by manual spraying during bunker silo loading. In the first year, LPUM1 was delivered at 10^6 CFU g^{-1} of fresh matter using sterile water as the carrier, while in the second year the inoculation dose (10^6 CFU g^{-1}) was suspended in cheese whey to improve cell dispersion and survival. Beyond its role as a delivery medium, cheese whey is characterized by a near-neutral pH, soluble carbohydrates, and low buffering capacity, properties that may facilitate early microbial implantation and metabolic activity when applied to complex plant matrices. In the present study, whey was therefore used as a functional carrier for the inoculant, with the aim of supporting microbial establishment without altering the intrinsic chemical composition of the tobacco biomass. Vacuum-packed bag models were prepared using the same biomass and inoculation protocols to monitor the anaerobic fermentation process under controlled laboratory conditions. These bags allowed for sequential sampling (0–150 days) and detailed profiling of microbial dynamics and metabolite production.

2.4. Sampling

2.4.1. Fermentation and maturation phase

During both cultivation years, the fermentation and maturation of cv. *Solaris* silages was monitored under anaerobic conditions on plant-scale, and on laboratory-scale in vacuum-packed bags stored under controlled conditions. More in detail, on laboratory scale,

changes in pH and lactic acid bacteria dynamics were monitored during the anaerobic phase at days 0, 30, 60, 90, 120, and 150 in untreated silage (Control_basic), silage supplemented with whey without LAB inoculation (Control_plus), and silages treated with *L. plantarum*, either directly (Treated_basic) or via cheese whey (Treated_plus).

2.4.2. Feed-out phase (post-opening of bunker silo)

In both years, ensiled cv. *Solaris* biomass (SiloSolaris) samples were taken from the silo surface every 30 days (n = 45) throughout the feed-out phase in order to monitor silage changes over time, following the sampling scheme shown in Fig. 3 (points a-e). Additional sampling points (x, x1; Fig. 3), were collected from the lateral sides of the bunker silo, where the biomass was less compacted than in the central zone and consequently may not have been fully preserved under anaerobic conditions.

2.5. Analytical determinations

2.5.1. Chemical and nutritional analyses

Chemical analyses that included the determination of dry matter (DM), organic matter, crude protein, ether extract, ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF) were performed according to official methods (AOAC, 2000) on SiloSolaris samples collected following the sampling scheme previously reported (Fig. 3). The total alkaloid content, expressed as g nicotine 100 g^{-1} DM, was quantified by the colorimetric method of Lidzey and Savage (1986), based on cyanogen chloride cleavage of the pyridine ring, with absorbance measured at 460 nm (Technicon AutoAnalyzer 2, Seal Analytical, UK). Moreover, on-field monitoring was carried out every 30 days to record temperature (mercury-glass thermometer) and pH (portable pH meter with penetrating electrode, Crison MM40, Alella, Spain).

2.5.2. Microbiological analyses

Microbiological counts were conducted both during vacuum-bag fermentation (days 0–150) and during the feed-out phase of bunker silo (post-opening, days 0–120). At each sampling time, 10 g of silage were aseptically collected and homogenized with 90 mL of sterile peptone water solution (0.1% w/v), using a Stomacher 400 Lab-blender (Seward Medical, London, UK) for 2 min. Serial decimal dilutions were prepared and plated in duplicate on selective culture media.

The following microbial groups were enumerated under specific culture conditions: i) lactic acid bacteria on de Man, Rogosa, and Sharpe (MRS) agar after anaerobic incubation at 30 °C for 48–72 h (AnaeroGen, Oxoid); ii) spore-forming *Clostridia* after heat treatment of diluted samples at 80 °C for 10 min to inactivate vegetative cells, followed by plating on Tryptose-Sulfite-Cycloserine (TSC) agar supplemented with selective additives and anaerobic incubation at 37 °C for 48 h; iii) yeasts and molds on Rose-Bengal Chloramphenicol Agar incubated aerobically at 25 °C for 5 days. The persistence of *L. plantarum* LPUM1 was verified by randomly isolating colonies from MRS plates at each sampling time, followed by purification and strain-level identification by RAPD-PCR, using previously validated strain-specific profiles.

2.5.3. Fungal isolation and identification

To characterize the microbiological evolution of tobacco cv. *Solaris* silage, fungal communities were monitored through the isolation and identification of the species occurring during the feed-out phase. Representative samples (n = 21) from each sampling point (Fig. 3) of the ensiled biomass, covering three periods (days 0–30, 31–60, and 61–90; total sub-samples n = 63), were plated on Potato Dextrose Agar (PDA) supplemented with ampicillin (100 μg mL^{-1}) to inhibit bacterial growth. Single fungal colonies were culture-purified on the same medium, and subjected to DNA extraction and PCR to amplify the interspace transcribed regions (ITS) using primers ITS4 and ITS5, as previously reported (White et al., 1990). PCR was carried out using premixed EconoTaq plus 2X Master Mix (Biosearch Technologies) according to the manufacturer instructions. The obtained amplicons were subjected to Sanger sequencing using primers ITS4 and ITS5, and the sequences obtained were subjected to BLASTn search on the ITS database available in GenBank for taxonomic classification (last accession October 2024).

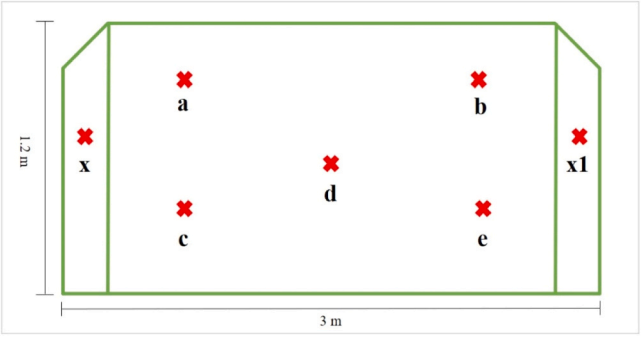

Fig. 3. Schematic representation of the SiloSolaris bunker silo showing the front surface sampling points design adopted for chemical, nutritional, and microbiological analyses.

2.5.4. Determination of fermentation metabolites

To further elucidate the fermentative pathways and stabilization mechanisms of cv. *Solaris* silage, a targeted set of complementary chemical analyses was conducted on samples obtained from the laboratory-scale vacuum-packed ensiling trials. These determinations were specifically designed to provide a mechanistic interpretation of the microbial dynamics and pH evolution described in the preceding sections. Organic acids (lactic, acetic, propionic and butyric acids) and ethanol were quantified in silage extracts collected at 0, 15, 30, 60 and 150 days of anaerobic storage from all experimental conditions (Control_basic, Control_plus, Treated_basic and Treated_plus). Briefly, 20 g of silage were homogenized with 180 mL of distilled water, filtered through Whatman No. 1 filter paper, and centrifuged at 10,000 × g for 10 min. The resulting supernatants were filtered through 0.22 μm membrane filters and analyzed by high-performance liquid chromatography (HPLC) equipped with a refractive index detector and an ion-exclusion column. Separation was performed using 0.005 M H_2SO_4 as the mobile phase at a flow rate of 0.6 mL min^{-1} . Proteolysis during ensiling was assessed by measuring ammoniacal nitrogen (NH_3–N), expressed as a percentage of total nitrogen, using the phenol–hypochlorite colorimetric method. Analyses were carried out on silage samples collected at 0, 30 and 150 days of anaerobic fermentation. To support the interpretation of the whey-mediated inoculation strategy, residual lactose was determined in whey-supplemented silages (Control_plus and Treated_plus) at 0 and 15 days of fermentation by enzymatic spectrophotometric assay. This analysis was used to monitor the early utilization of the carrier-derived fermentable substrate in relation to microbial implantation and fermentation kinetics. All determinations were performed in triplicate.

2.6. Statistical analysis

Morphological and productive traits of *Nicotiana tabacum* cv. *Solaris* plants were subjected to one-way ANOVA. Pairs of means were compared using the Tukey HSD test. These analyses were performed using Origin Pro software (OriginLab Corporation, Northampton, MA, USA). Chemical composition, temperature, pH, and total alkaloid content of cv. *Solaris* ensiled biomass were processed by analysis of variance through the GLM procedure (IBM SPSS Statistics Data Editor ver. 25, Chicago, IL, USA). The evolution of microbial counts and pH values during fermentation and storage were statistically evaluated by one-way or repeated-measures ANOVA followed by Dunnett's or Šidák's post-hoc tests, enabling regression models and correlation analyses between acidification dynamics and microbial ecology. Fermentation metabolites were statistically evaluated by one-way ANOVA followed by Tukey's post-hoc test (GraphPad Software, San Diego, CA, USA). All data were expressed as mean ± standard error (SEM) and differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Morphological traits of tobacco cv. Solaris

The growth of tobacco cv. *Solaris* was evaluated measuring fresh biomass, plant height, number of leaves per area unit, leaf dry matter content, and leaf area index (Table 1). Plants grown during the first year of cultivation, with a density of 36,000 plants ha^{-1} , reached a calculated fresh biomass production of 48.4 t ha^{-1} , which was significantly higher than that obtained during the second year (23.9 t ha^{-1} at 32,000 plants ha^{-1}). These values are consistent with those reported by Ren et al. (2021) for tobacco crops cultivated with biochar, but lower than previous experimental findings where average fresh biomass reached approximately 70 t ha^{-1} (Grisan et al., 2016; Fatica et al., 2019). The literature confirms a marked variability in tobacco production, as also observed by Berbeć and Matyka (2020) in Eastern Poland, where whole-crop fresh matter ranged from 34.8 to 62.3 t ha^{-1} with an average of 53.2 t ha^{-1} .

Leaf-related parameters followed a similar trend. The number of leaves per square meter decreased from 187.6 in year I to 88.6 in year II, and the leaf dry matter content declined from 29.3 g 100 g^{-1} to 20.2 g 100 g^{-1} over the same period, with both differences statistically significant ($p < 0.01$). Despite this reduction, no significant variations were observed in the LAI (Table 1). These values are in line with the variability reported in the literature for flue-cured tobacco, where LAI ranged from 0.61 to 4.41, with an average of 2.57 (Li et al., 2024a). Plant height was unaffected by cultivation year, confirming the limited sensitivity of this parameter to the experimental field conditions.

Being that the same *Solaris* cultivar was used in both years, the contrasting results between the two cultivation years can be primarily attributed to the environmental variability. Temperature patterns during the growing periods (May-October) were broadly similar in both years, with maximum values exceeded 30° C in June, July, and August, a threshold above which the photosynthetic activity of tobacco is progressively inhibited (Sifola et al., 2022). However, the main climatic difference between the two cultivation

Table 1
Morphological traits of cv. *Solaris* plants measured during the two-year period (n = 45).

	Year I	Year II	DF	F-value	*p* value
Fresh biomass, t ha^{-1}	48.4[a]	23.9[b]	2	6.2197	0.009
Plant height, cm	136.8	141.1	2	1.77	0.200
Leaves, n per m^{-2}	187.6[a]	88.6[b]	2	10.23105	0.005
Leaves dry weight, g/100 g	29.3[a]	20.2[b]	2	8.04292	0.003
Leaf area index (LAI)	3.45	2.58	2	0.72571	0.500

Different superscript letters ([a,b]) indicate significant differences ($p < 0.05$). All values are expressed as means and pooled SEM.

years was represented by rainfall distribution. Total precipitation during the cultivation period (May-October) was 168 mm in year I and 508 mm for year II, indicating substantially wetter conditions in the second year. Moreover, vegetative development was further constrained by excessive rainfall in year II, which caused temporary waterlogging due to poor drainage, both after transplanting and floral apexes collections, affecting plant growth and biomass production. The different plant density may have played a secondary role in modulating biomass and leaf development, highlighting the complex interaction between plant density, climatic variability, and biomass yield in energy tobacco cultivation. Accordingly, tobacco cv. *Solaris* can represent a potentially valuable biomass source for energy purposes, although its productivity is strongly influenced by environmental conditions and management practices. Enhanced yields could be achieved through advanced strategies such as optimized plant density, balanced fertilization regimes, and irrigation schedules tailored to local climatic variability (Berbeć and Matyka, 2020).

3.2. Chemical, technological and microbiological assessment of cv. Solaris silage

3.2.1. In vitro traits of Lactiplantibacillus plantarum LPUM1

The *in vitro* characterization of *L. plantarum* LPUM1 demonstrated a pronounced multifunctional profile, confirming its potential as an effective silage inoculant. The strain exhibited broad-spectrum antimicrobial activity against fungal and bacterial targets typically associated with silage spoilage. Agar well diffusion assays reported in Fig. 4a revealed clear inhibition halos (9–15 mm) around the LPUM1 cell-free supernatant (CFS) after 72 h of incubation at 28 °C when tested against *Aspergillus flavus*, *Fusarium graminearum*, and *Penicillium roqueforti*.

Under anaerobic conditions, LPUM1 significantly inhibited the growth of *Clostridium butyricum* and *C. tyrobutyricum*, confirming its capacity to suppress clostridial proliferation in low-oxygen environments (Fig. 4b).

The observed antagonistic behavior confirms previous findings demonstrating the strong inhibitory potential of *L. plantarum* isolates toward a wide range of spoilage and pathogenic microorganisms, mainly through the synergistic action of organic acids, hydrogen peroxide, and low-molecular-weight antimicrobial molecules (Tremonte et al., 2017). Likewise, Tremonte et al. (2017) reported that metabolic products from *L. plantarum* can effectively inhibit undesirable microbiota in fermented systems, highlighting the relevance of strain-dependent metabolic traits. More recent comparative studies on *L. plantarum* biodiversity and technological functionality further emphasized the ecological adaptability and bioprotective aptitude of strains isolated from spontaneous fermentations, supporting the selection of LPUM1 as a technologically valuable and resilient inoculant (Iarusso et al., 2025).

3.2.2. Chemical and physico-chemical characterization of cv. Solaris ensiled biomass

The chemical components of cv. *Solaris* ensiled biomass reported in Table 2 did not show differences ($p > 0.05$) between years. Nevertheless, the pH of SiloSolaris in year II was significantly lower ($p < 0.01$) compared to the value recorded in year I (Table 2).

Generally, the ensiling process aims to preserve the nutritional composition of the biomass during the anaerobic fermentation and buffer seasonal variations (Ramos et al., 2016). Although the nutritional composition of tobacco cv. *Solaris* silage did not differ significantly between years (Table 2), the significant decrease of average pH and the tendential decrease ($p = 0.093$) of temperature values observed in SiloSolaris in year II, suggest an improvement in the storage conditions. Comparing the chemical components of SiloSolaris with previously reported data, it is worth noting that DM and organic matter from both cultivation years were slightly lower than the values reported by Fatica et al. (2021), although crude protein content was similar, while ether extract content was halved compared to the previous studies (Fatica et al., 2021), likely due to the earlier harvest stage of the whole plant in the present study. The higher ash content observed may be related to the type of bunker silo used, *i.e.*, ground-based versus reinforced concrete (Fatica et al., 2021). Indeed, ground-based silages are more affected by soil contamination during filling and packing due to direct contact with soil

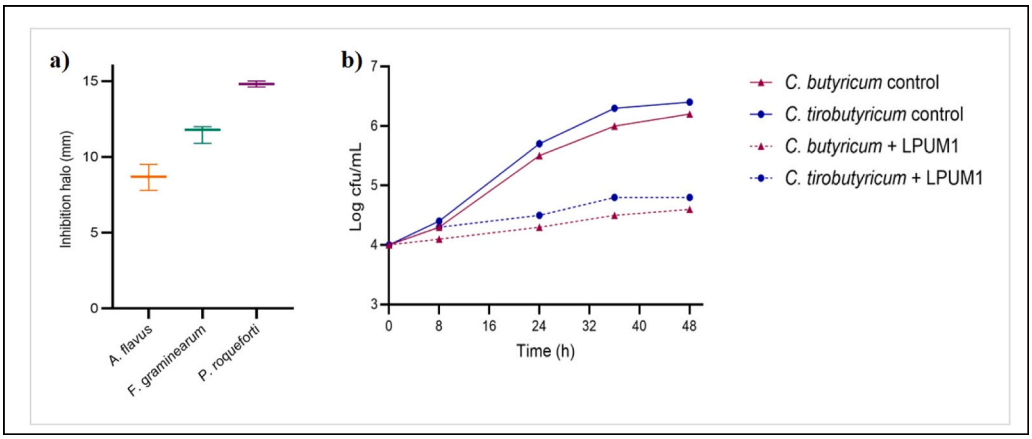

Fig. 4. Antifungal activity (mean inhibition halos ±SD) of *Lactiplantibacillus plantarum* LPUM1 cell-free supernatant against silage-associated molds **(a)**; inhibitory effect of *L. plantarum* LPUM1 on clostridial growth under anaerobic conditions. Clostridial monoculture (controls, solid lines); clostridial co-culture with *L. plantarum* LPUM1 (dashed lines) **(b)**.

Table 2
Chemical and physico-chemical parameters of tobacco cv. *Solaris* ensiled biomass (g 100 g^{-1} DM, unless otherwise indicated, n = 45).

	Year I	Year II	SEM	*p* value
Dry matter (g 100 g^{-1})	19.1	22.5	2.037	0.350
Organic matter	83.3	85.3	0.897	0.268
Crude protein	16.8	17.0	0.268	0.765
Ether extract	5.44	4.51	0.160	0.054
Ash	16.7	14.7	0.897	0.268
NDF	45.6	48.3	1.882	0.422
ADF	40.1	34.4	1.227	0.080
Total alkaloids (g nicotine 100 g^{-1} DM)	0.46	0.11	0.178	0.303
Temperature (°C)	13.5	8.00	1.275	0.093
pH	5.30[a]	5.03[b]	0.011	0.003

SEM = standard error of the mean; NDF = neutral detergent fiber; ADF = acid detergent fiber. Different superscript letters ([a,b]) indicate significant differences ($p < 0.05$). All values are expressed as means and pooled SEM.

and the transfer of particles by machinery (Hoffman, 2005; Martens et al., 2018). Tobacco cv. *Solaris* cultivated in the Benevento area also showed higher NDF and ADF contents compared to previous cultivation in the Chieti province (Fatica et al., 2021). Average pH values recorded in year II of cultivation were consistent with those reported by Fatica et al. (2021), while total alkaloids expressed as nicotine decreased markedly, by −66% in year I and by −91% in year II, compared to the same previous report. It should also be emphasized that plant genetics, field and crop conditions, insect predation, mechanical injury, and agronomic practices can influence the biosynthesis and accumulation of tobacco alkaloids, of which nicotine accounts for approximately 90% (Wang et al., 2015). Nevertheless, the alkaloid content in SiloSolaris, expressed as nicotine, was lower than that of traditional smoking tobacco cultivars, which range from 2.5% to 4.3%, and may reach 5–7% in heavy-bodied tobacco (Tassew and Chandravanshi, 2015; Mahbub et al., 2023).

3.2.3. *Evolution of fermentative parameters on silage stability*

The effect of inoculants on the microbial ecology of silage may vary according to the inoculant species and the type of crop ensiled (Dunière et al., 2013; McAllister et al., 2018), as fungal succession is strongly shaped by fermentation-driven ecological filters such as pH decline, organic acid accumulation, substrate availability, and oxygen exposure during feed-out. Microbiological analyses conducted during both the ensiling and feed-out phases confirmed the functional effectiveness of *L. plantarum* LPUM1 as a silage inoculant, particularly when applied using whey as a carrier, which improved microbial dynamics and fermentation stability compared to treatments without whey supplementation.

The main fermentation parameters are reported in Fig. 5, which illustrates the temporal evolution of pH over 150 d (Fig. 5a), and the dynamics of lactic acid bacteria populations (Fig. 5b) across four ensiling conditions. Clear differences emerged in acidification kinetics and stabilization among treatments.

The untreated silage (Control_basic) showed only a modest pH decline, stabilizing above 5.6, while the addition of whey without inoculation (Control_plus) induced a slightly greater pH reduction. Inoculation with *L. plantarum* LPUM1 (Treated_basic) led to a more pronounced acidification, stabilizing around pH 5.4–5.5 (Fig. 5a). The strongest effect was observed in Treated_plus, where inoculation

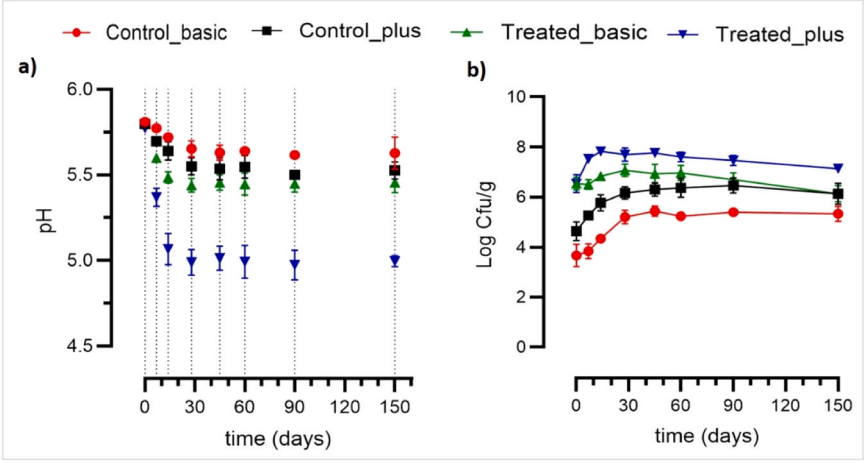

Fig. 5. Temporal dynamics of pH **(a)** and lactic acid bacteria populations **(b)** during anaerobic storage of *Solaris* biomass in four ensiling conditions: untreated control without whey (● Control_basic), untreated control supplemented with whey (■ Control_plus), silage inoculated with *L. plantarum* LPUM1 without whey (▲ Treated_basic), and silage inoculated with *L. plantarum* LPUM1 and supplemented with whey (▼ Treated_plus). Error bars represent standard deviations (n = 3).

of LPUM1 combined with whey supplementation produced the fastest and deepest pH decline, reaching about pH 5.0 within 15 days and maintaining this low level throughout 150 days. The temporal evolution of LAB populations revealed significant differences between treatments ($p < 0.05$, Fig. 5b). Control_basic exhibited the slowest and lowest increase, reaching a plateau at ~5.0 log CFU g^{-1}. Control_plus supported a faster increase to about 6.0 log CFU g^{-1}, which remained stable over storage. Treated_basic reached slightly higher values (about 6.0–6.3 log CFU g^{-1}), but without significant divergence from Control_plus after day 30. By contrast, Treated_plus showed the most rapid increase, offering a promising pathway toward sustainable and high-quality silage production.

Overall, these data demonstrate that while inoculation alone improves fermentation quality, the most consistent and durable fermentative outcome was observed when *L. plantarum* LPUM1 was applied in combination with whey supplementation. Under these synergistic conditions the coordinated use of a selected inoculant and a fermentable carrier promoted a faster establishment of lactic acid bacteria, sustained their dominance, and accelerated acidification, resulting in a more stable preservation trajectory. From a technological perspective, the results underscore the process-level advantage of integrating a competitive microbial culture with a nutrient-rich agro-industrial by-product, rather than the effect of inoculation alone. This interpretation is consistent with current evidence indicating that microbial functionality in fermented systems is strongly modulated by substrate availability and carrier-mediated implantation, as reported for a range of food and feed fermentations (Lombardi et al., 2024, 2025).

To assess aerobic stability, the silages treated with *L. plantarum*, either applied directly (Treated_basic) or delivered through cheese whey (Treated_plus), were monitored for pH evolution and LAB dynamics following the silo opening. The results are summarized in Fig. 6, which reports the changes in pH (Fig. 6a) and the evolution of LAB populations (Fig. 6b) during aerobic exposure. Specifically, Treated_basic batch exhibited a progressive increase in pH from 5.45 at the onset of aerobic exposure to 5.72 by 90 d, stabilizing around 5.65–5.74 at 120 d. In contrast, Treated_plus maintained markedly lower and more stable values, fluctuating narrowly around pH 4.95–5.10 throughout the 120 d period. This behavior supports the interpretation that whey-mediated delivery of the inoculant enhanced the resilience of the fermentative system during oxygen exposure, a response commonly associated with improved aerobic stability in silages (Malos et al., 2025). LAB populations showed divergent trajectories: in Treated_basic, counts declined from initial values of about 6.0 log CFU g^{-1} to 4.5–5.0 log CFU g^{-1} by 120 d, reflecting a loss of microbial viability and competitive dominance under aerobic conditions. Conversely, samples from Treated_plus batches maintained significantly higher LAB populations, starting at about 7.0 log CFU g^{-1} and remaining above 6.4 log CFU g^{-1} after 120 d. These differences were statistically significant at all time points ($p < 0.01$), underscoring the enhanced persistence and stability of the LAB community when the inoculum was delivered via whey. Similar trends were reported when additive and/or inoculant combinations sustained LAB activity and effectively suppressed undesirable microbial competitors during aerobic exposure (Auerbach et al., 2020; Li et al., 2024b).

The integrated interpretation of these data indicates that the additive effect of inoculation and whey supplementation extended beyond the anaerobic phase, providing a measurable advantage in aerobic stability. While Treated_basic supported a transient improvement in fermentation quality, it was unable to prevent pH elevation and microbial decline after feed-out phase. By contrast, Treated_plus maintained both a stable acidic environment and robust LAB populations, thereby minimizing the risk of spoilage microorganism proliferation. These findings suggest that inoculant-carrier strategies may enhance aerobic stability by sustaining metabolically active LAB communities, thereby mitigating oxygen-induced metabolic shifts and limiting the proliferation of undesirable competitors such as yeasts and molds (Guo et al., 2018; Drouin et al., 2020; Muck et al., 2025). Microbiological analyses performed during both the ensiling and feed-out phase further confirmed the functional effectiveness of *Lactiplantibacillus plantarum* LPUM1 as a silage inoculant. Strain-specific RAPD-PCR consistently identified LPUM1 among the lactic acid bacteria recovered from the silage matrix, confirming its persistence throughout the entire process under pilot-scale conditions.

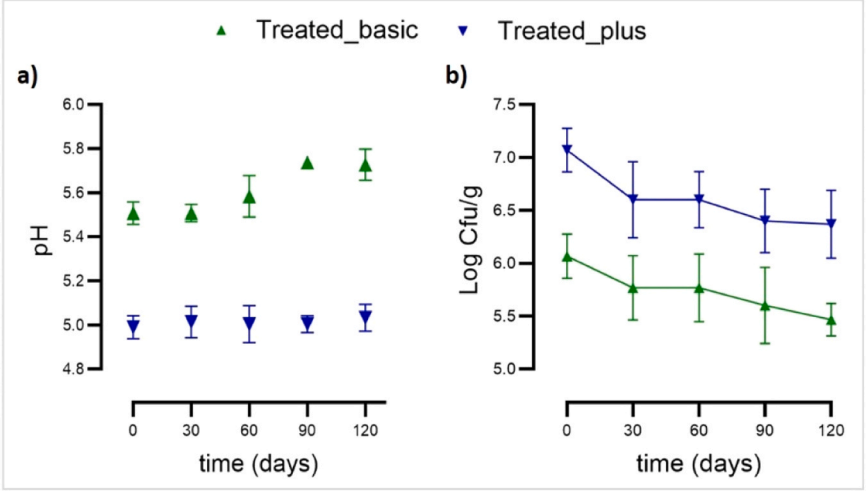

Fig. 6. Aerobic stability of *L. plantarum*-inoculated silages after the silo opening: pH evolution **(a)** and lactic acid bacteria dynamics **(b)** during 120 d of aerobic exposure (mean ± SD) in silage inoculated directly with *L. plantarum* (▲ Treated_basic) and silage inoculated with *L. plantarum* delivered through cheese whey (▼ Treated_plus).

The evolution of spoilage microbiota during aerobic exposure was assessed by monitoring eumycetes (yeasts and molds) and *Clostridia* in the two inoculated silages, with or without whey supplementation. Results are reported in Fig. 7, which illustrate clostridial counts (Fig. 7a) and eumycete dynamics (Fig. 7b) over 120 d. Significant differences were detected among the batches in clostridial populations (Fig. 7a). In Treated_basic, clostridial counts remained detectable across the 120 d period, with values fluctuating between 3.5 and 4.5 log CFU g^{-1}, suggesting incomplete suppression and potential residual activity. In contrast, Treated_plus showed markedly reduced clostridial levels, consistently close to or below 1.5 log CFU g^{-1} throughout the trial, with minimal variation over time. This suppression can be attributed to both the lower pH maintained during aerobic exposure and the competitive dominance of LAB, as previously noted in inoculated silages with enhanced stability (Akhtar et al., 2025; Gou et al., 2025). Moreover, samples from the Treated_basic batch were characterized by a continuous and significant increase in fungal population counts up to day 60, after which values remained relatively constant, indicating a persistent fungal presence during prolonged anaerobic storage (Fig. 7b). In contrast, the Treated_plus samples exhibited an early arrest of mold growth followed by almost complete inhibition (Fig. 7b).

These results are consistent with previous reports showing that strong lactic acid production, sustained low pH, and active lactic acid bacterial metabolism effectively suppress yeast and mold proliferation, thereby enhancing aerobic stability of silages (Azevedo and Gierus, 2025). Taken together, the results of Fig. 7 confirm that whey-mediated inoculation not only promoted LAB persistence and stabilized pH (Figs. 5 and 6), but also exerted a decisive inhibitory effect on spoilage microorganisms. The reduction in eumycetes and *Clostridia* in Treated_plus underscores the ecological advantage of coupling a selected inoculant with a nutrient-rich carrier, thereby extending the protective effect from anaerobic preservation to aerobic stability. This finding is of technological relevance, as aerobic deterioration during feed-out represents a major source of nutrient loss and hygienic risk in silage-based feeding systems (Guo et al., 2025; Liu and Zhang, 2025; Muck et al., 2025). Accordingly, in the Treated_basic batch, where LPUM1 was applied directly to the biomass without whey as a carrier, conspicuous white mold growth was observed on most of the exposed surface during the first half of the sampling period under aerobic conditions (Fig. 8a-c).

ITS analysis of purified fungal cultures of the first and second sampling periods, and in all sampled areas (Fig. 3), revealed the presence of a fungus belonging to the *Paecilomyces* genus, with the sequences obtained with primer ITS5 sharing high similarity with *P. niveus*, and that obtained with the primer ITS4 sharing high similarity with *P. penicilliformis* (Table 3); both sequences share high similarity (E-value = 0.0) also with *P. fulvus*, which in BLASTn analysis was the third hit. This isolate is indicated as *Paecilomyces* spp. The isolated mold develops with abundant white mycelium also on PDA media (Fig. 8d), and microscopic analysis confirms a highly irregularly branched mycelium with septa and chlamydospores (Fig. 8e-g), and conidiophores with phialides forming conidia and hyaline conidia, which have a classical ellipsoidal or pyriform shape and are produced in chains (Fig. 8h).

In both sampled periods and only for the sampled points a, x, and x1 (Fig. 3), two other fungal isolates were identified: the fungus isolated in the first sampled period belongs to the *Penicillium* genus, with the sequencing carried out with primer ITS5 returning *P. expansum* (E-value = 0.0), and that carried out with primer ITS4 returning a different species, *P. robsamsonii* (E-value = 0.0). The fungus isolated in the second sampled period share similarity with *Geotrichum pseudocandidus* for the sequencing carried out with primer ITS5, and with *Geotrichum pandrosion* for the sequencing carried out with primer ITS4. In this case the BLASTn analysis returned E-values of 2e−140 and 4e−161, indicating that most likely this isolate is a novel *Geotrichum* species or a *Geotrichum* spp. for which ITS regions have not been deposited yet in GenBank database. In the third sampled period, the widely spread-out white mold *Paecilomyces* spp. could not be isolated, and an overall increase of fungal diversity was found. In particular, in several sampled areas were detected the same *Geotrichum* spp. isolate described before, and the species *Talaromyces guatemalensis*. Moreover, in two other sampled areas at

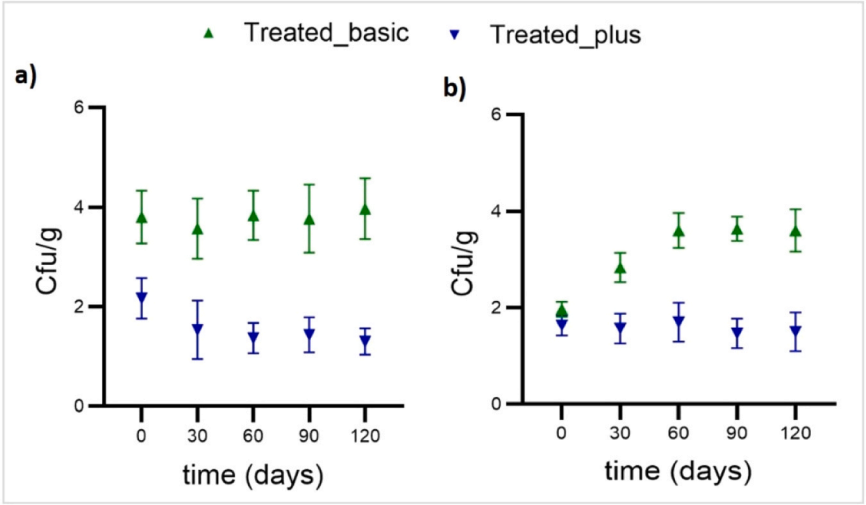

Fig. 7. Time course of undesirable microorganisms' detection during the aerobic stability phase of SiloSolaris inoculated with *L. plantarum* LPUM1. Dynamics of *Clostridium* spp. over 120 d of aerobic exposure **(a)**; evolution of eumycete populations (yeasts and molds) **(b)**. Bars indicate standard deviations (n = 3).

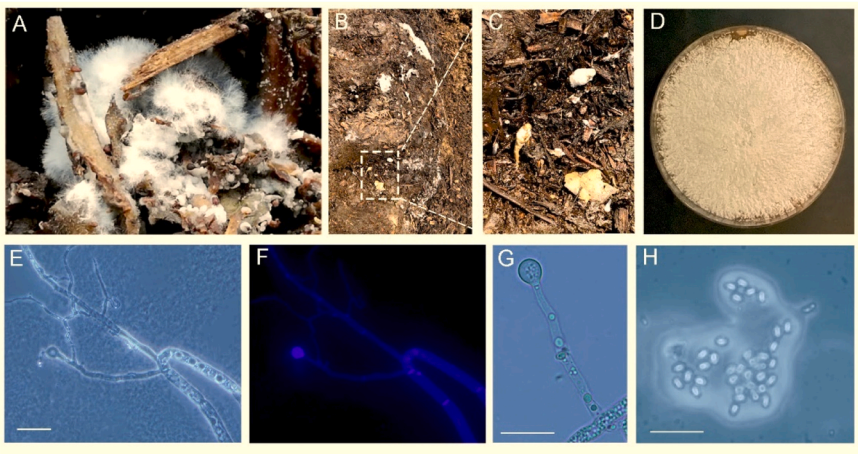

Fig. 8. Abundant contaminations by the white mold *Paecilomyces* spp. on the surface of the SiloSolaris **(a,b)**; magnification of the picture in B showing a fungal fruiting body (white arrow) **(c)**; SiloSolaris-isolated *Paecilomyces* spp. on PDA agar after 4 days of growth at 25°C **(d)**; Differential Interference Contrast **(e)** and calcofluor white staining **(f)** of *Paecilomyces* spp. branching hyphae, with the white arrow indicating a chlamydospore; note in F the septa, and a bright chlamydospore whose signal intensity likely indicates chitin accumulation; magnification of a chlamydospore **(g)**; conidiophores with phialides forming conidia and conidia **(h)**.

Table 3
ITS analyses result from purified fungal cultures during the different sampling periods.

Sampling	Sampling point	Seq. primer	E-value best hit	% of identity	Closest fungal genus/species (ITS Database NCBI)	Accession	Note
First	a, b, c, d, e, x,	ITS5	0.0	99.64	*Paecilomyces niveus*	NR_144910.1	
	x1	ITS4	0.0	97.94	*Paecilomyces penicilliformis*	NR_191058.1	*P. niveus* is the second hit
	a	ITS5	0.0	99.46	*Penicillium expansum*	NR_077154.1	
		ITS4	0.0	98.03	*Penicillium robsamsonii*	NR_144866.1	*P. expansum* is the third hit
Second	a, b, c, d, e, x,	ITS5	0.0	99.64	*Paecilomyces niveus*	NR_144910.1	
	x1	ITS4	0.0	97.94	*Paecilomyces penicilliformis*	NR_191058.1	*P. niveus* is the second hit
	a	ITS5	2e−140	92.82	*Geotrichum pseudocandidus*	NR_163519.1	
		ITS4	4e−161	96.76	*Geotrichum pandrosion*	NR_189975.1	*G. pseudocandidus* is the second hit
Third	b, c, d	ITS5	6e−140	92.80	*Geotrichum pseudocandidus*	NR_163519.1	
		ITS4	4e−161	96.76	*Geotrichum pandrosion*	NR_189975.1	*G. pseudocandidus* is the second hit
	a, c, d, e,	ITS5	0.0	97.20	*Talaromyces guatemalensis*	NR_191090.1	
		ITS4	0.0	97.60	*Talaromyces guatemalensis*	NR_191090.1	
	x	ITS5	0.0	98.71	*Fusarium fujikuroi*	NR_111889.1	
		ITS4	0.0	99.60	*Fusarium concentricum*	NR_111886.1	*F. fujikuroi* is the second hit
	x1	ITS5	0.0	99.81	*Pseudallescheria ellipsoidea*	NR_130665.1	
		ITS4	0.0	100.00	*Pseudallescheria ellipsoidea*	NR_130665.1	

the edge of the ensiled biomass (x and x1 points), a species within the *Fusarium* genus was found, with *F. fujikuroi* and *F. concentricum* being the most closely related species, and the fungal species *Pseudallescheria ellipsoidea*. Note that for *T. guatemalensis* and *P. ellipsoidea* we were able to assign with high confidence the fungal species name because of the very high similarity displayed by the BLASTn analyses (Table 3).

The widespread presence of *Paecilomyces* spp. on the surface of the ensiled biomass exposed to the air is likely the result of several factors, such as the substrate complexity of *Nicotiana tabacum* cv. *Solaris* biomass, which is rich in carbonaceous compounds, structural fibers (NDF, ADF), and residual nitrogenous fractions that can support fungal growth. However, since only the external surface layer of the silage was affected by fungal growth and this portion was discarded before animal feeding, and considering that no fungi were isolated from the fermenting biomass core, the presence of *Paecilomyces* spp. did not represent a risk for animal health. Indeed, generally the upper sections of silos are more exposed to oxygen infiltration and, consequently, more susceptible to fungal contamination than the middle sections (Alonso et al., 2013).

The occurrence of fungi belonging to the genera *Penicillium*, *Geotrichum*, *Fusarium*, and *Pseudallescheria* during the feed-out phase can be interpreted as the outcome of process-related conditions rather than as an intrinsic limitation of the cv. *Solaris* biomass. Their detection was more evident under ensiling conditions associated with a less efficient fermentative performance, particularly in year I, when the starter culture was applied without whey as a carrier. In this context, acidification kinetics might have left ecological niches open to opportunistic molds with high metabolic versatility (*i.e.*, *Penicillium*, *Fusarium*), due to poor fermentation associated with

oxygen presence which could promote fungal proliferation (Alonso et al., 2013; Ávila and Carvalho, 2020; Lombardi et al., 2022). Rapid acidification driven by *L. plantarum* could be associated with a marked shift in microbial community structure and reduced diversity, whereas delayed acidification corresponds to a more heterogeneous microbiota (Yang et al., 2019).

The disappearance of *Paecilomyces* spp. in the final sampling period supports the progressive stabilization of the ensiling system during feed-out phase. This stabilization can be attributed to the combined effect of improved aerobic stability, depletion of readily available substrates at the silo face, and enhanced physical management practices, including compaction efficiency and controlled biomass removal. Consistently, pH evolution and microbial patterns (Fig. 5) supports these dynamics, while fermentation metabolites provide a quantitative biochemical context. Lactic acid was the predominant fermentation product in all silages, although differences were observed among treatments in both accumulation kinetics and final concentrations (Table 4).

After 15 days of anaerobic storage, lactic acid concentration in Treated_plus silages was significantly higher ($p < 0.05$) than the values measured in the other silages (Table 4). Similarly, at 150 days Treated_plus silages exhibited the highest lactic acid concentration, followed by Treated_basic, Control_plus, and Control_basic, respectively. Acetic acid concentrations at the end of ensiling ranged from 12.4 to 18.9 g kg^{-1} DM across treatments, with slightly higher mean values in whey-supplemented silages, although these differences were not statistically significant ($p > 0.05$). Propionic acid was detected only in trace amounts (<1.5 g kg^{-1} DM) in all treatments. Butyric acid, used as an indicator of clostridial activity, remained below the detection limit (<0.5 g kg^{-1} DM) in Treated_plus silages throughout the storage period, whereas low but detectable concentrations were occasionally observed in Control_basic and Treated_basic at 60 and 150 days, in agreement with the clostridial counts reported in Fig. 7a. Ethanol concentrations remained low (< 6 g kg^{-1} DM) in all treatments, indicating the absence of excessive yeast-driven fermentation. Proteolysis, expressed as ammoniacal nitrogen (NH_3–N) as a percentage of total nitrogen, increased during ensiling in all treatments but followed distinct trajectories (Table 4). At the end of the anaerobic phase, NH_3–N remained lowest in Treated_plus, whereas progressively higher values were recorded in Treated_basic, Control_plus, and Control_basic, respectively. In whey-supplemented silages residual lactose significantly decreased ($p < 0.01$) in Treated_plus silage, compared to Control_plus. This differential lactose utilization coincided with the earlier establishment of lactic acid bacteria and higher lactic acid accumulation observed in Treated_plus silages. Taken together, these data indicate that the combined application of *Lactiplantibacillus plantarum* LPUM1 and whey was associated with a more pronounced lactic acid-driven fermentation, reduced proteolysis, and limited formation of undesirable metabolites. This biochemical profile is consistent with the improved anaerobic and aerobic stability observed in Treated_plus silages and supports the technological relevance of whey-mediated inoculation for the stabilization of cv. *Solaris* biomass. The effectiveness of whey-mediated inoculation observed in this study may be partially attributed to the characteristics of whey used. In particular, the combined presence of readily fermentable soluble carbohydrates and the relatively low buffering capacity, compared to fresh plant biomass, can support the rapid activation of lactic acid bacteria during the early stages of fermentation, especially when applied to structurally complex substrates, such as tobacco.

4. Conclusions

This study demonstrates that *Nicotiana tabacum* L. cv. *Solaris* can be effectively valorized as a non-conventional forage through ensiling practice. When harvested at appropriate physiological maturity and subjected to controlled anaerobic fermentation, the resulting silage showed a balanced chemical composition, with appreciable crude protein content, adequate levels of structural carbohydrates, and consistently low alkaloid concentrations, supporting its suitability for ruminant feeding.

The ensiling process was affected by high buffering capacity of the biomass and the limited availability of readily fermentable carbohydrates, which may hinder spontaneous acidification. However, the application of a *Lactiplantibacillus plantarum* inoculant, particularly when delivered via cheese whey, improved fermentation performance and silage quality.

Overall, the results support the practical feasibility of using cv. *Solaris* silage in ruminant nutrition. They also highlight the relevance of integrating agronomic and microbiological strategies to enhance biomass valorization and promote circular agriculture. Similar approaches could also be applicable to other underutilized or non-traditional biomass resources.

CRediT authorship contribution statement

Silvia Jane Lombardi: Writing – review & editing, Methodology, Formal analysis, Data curation. **Raffaello Castoria:** Writing – review & editing. **Giuseppe Ianiri:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Stefano Marino:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Antonella Fatica:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Elisabetta Salimei:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Francesco Fantuz:** Writing – review & editing, Methodology. **Massimo Zuin:** Writing – review & editing. **Patrizio Tremonte:** Writing – review & editing, Methodology, Formal analysis, Data curation.

Informed Consent Statement

Not applicable.

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Table 4
Lactic acid, acetic acid, butyric acid, ethanol and ammoniacal nitrogen (NH_3–N) in cv. *Solaris* silages subjected to different ensiling strategies during anaerobic storage. Values are expressed on a dry matter (DM) basis and reported as mean ± SD (n = 3).

	Time (d)	Control_basic	Control_plus	Treated_basic	Treated_plus
Lactic acid (g kg^{-1} DM)	15	21.4 ± 1.7 ^{c}	28.9 ± 1.9 ^{b}	31.6 ± 2.1^{b}	42.3 ± 2.8 ^{a}
	150	36.5 ± 2.6 ^{c}	44.2 ± 3.1 ^{b}	48.7 ± 2.9 ^{b}	61.8 ± 3.4 ^{a}
Acetic acid (g kg^{-1} DM)	150	12.4 ± 1.3	17.8 ± 1.9	15.6 ± 1.6	18.9 ± 2.1
Butyric acid (g kg^{-1} DM)	60	1.8 ± 0.3 ^{a}	< 0.5 ^{b}	1.2 ± 0.2 ^{a}	< 0.5 ^{b}
	150	2.1 ± 0.4 ^{a}	< 0.5 ^{b}	1.6 ± 0.3 ^{a}	< 0.5 ^{b}
Ethanol (g kg^{-1} DM)	150	5.2 ± 0.6 ^{a}	4.1 ± 0.5^{b}	3.3 ± 0.4^{c}	2.9 ± 0.3^{d}
NH_3–N (% total N)	30	12.6 ± 1.0 ^{a}	10.1 ± 0.8 ^{b}	9.4 ± 0.7 ^{b}	6.8 ± 0.5 ^{c}
	150	16.2 ± 1.3 ^{a}	13.4 ± 1.1 ^{b}	12.7 ± 0.9 ^{b}	8.9 ± 0.6 ^{c}
Residual lactose (g kg^{-1} DM)	0	–	21.4 ± 2.2 ^{a}	–	21.3 ± 2.1 ^{a}
	15	–	11.8 ± 1.3 ^{a}	–	4.6 ± 0.7 ^{b}

Control_basic: untreated silage without whey; Control_plus: untreated silage supplemented with whey; Treated_basic: silage inoculated with *Lactiplantibacillus plantarum* LPUM1; Treated_plus: silage inoculated with *L. plantarum* LPUM1 delivered through cheese whey. Different superscript letters (^{a,b,c}) indicate significant differences ($p < 0.05$). All values are expressed as means and pooled SEM. "< 0.5" indicates values below the analytical detection limit.

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Declaration of Competing Interest

Each of the authors confirms that this manuscript has not been previously published and is not currently under consideration by any other journal, all of the authors have approved the contents of this paper and have agreed to the Animal Feed Science and Technology submission policies. Additionally, to the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

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