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Contents

Paper 1: The suitability of quinoa as an alternative dry season crop to rice for growth in climate affected areas of the Mekong River Delta Vietnam

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Abstract

Saline intrusion, drought and water shortages are causing rice production losses in the Mekong River Delta dry season. Quinoa is a water use efficient halophyte that could be an alternative crop option to rice or fallow in the dry season. The novelty of growing quinoa in the delta requires an understanding of variety selection and crop optimisation in conditions experienced in the dry season. A greenhouse trial was conducted in 2022 to screen for saline tolerant and water efficient varieties (2-want, Sluga, Titicaca, 42- test, Atlas). The higher yielding varieties, Titicaca and 2-want, had lower biomass and improved water use efficiency in saline and water limited conditions. A 2023 field trial utilising these varieties investigated their response to nitrogen fertiliser rates (0, 40, 80 and 120 kg N/ha). Nitrogen fertiliser rates of 120 kg N/ha produced the greatest biomass and yield, however lodging occurred, and the 2-want variety recorded negligible yield. A 2024 sowing density trial tested if lodging could be decreased with higher planting density of Titicaca. Sowing densities between 15 plants/m^2 and 50 plants/m^2 had no significant effect on lodging rate. This trial also experienced an uncharacteristic heatwave during anthesis causing no measurable yield. Though these experiments identify Titicaca as suitable variety of quinoa based on tolerance to salinity and agronomic performance, sensitivity to temperature has been identified as a risk factor. Manipulating the growing calendar so that saline and water limited tolerant varieties like Titicaca avoid peaks in temperature during anthesis may provide farmers with a suitable dry season alternative crop to rice.

1. Background

The Mekong River Delta (MRD) is a significant agricultural region of Vietnam producing over 50% of the country’s rice (GSO, 2021). Extensive investment in irrigation infrastructure and the heightening of dikes in 2000 allowed triple rice production to dominate land use and contribute to Vietnam’s shift to net rice exportation (Tran et al., 2018, Sakamoto et al., 2009). However, the MRD is ranked highly vulnerable to climate change (WorldBank, 2021) with dry season saline intrusion and water shortages continuously threatening rice production (Nghia et al., 2024, Loc et al., 2021, Dang et al., 2020). The land use policies of the peri coastal regions of the MRD had designated some at risk areas as triple rice (three rice crops per year) (Nguyen et al., 2020b). In these areas, salinity intrusion events in 2016, 2020 resulted damage to rice in more than 200,000 and 40,000 ha respectively (WorldBank, 2017; Nghia et al., 2024). The severely affected province of Sóc Trăng has experienced rice yield decline of up to 4 t/ha due to salinity stress (Minh et al., 2022). In response to multiple severe droughts and salinity intrusion events, there was a focus on management and policy changes to mitigate these hazardous events (Nghia et al., 2024). A changed land use policy in 2000 identified the need to adapt to changing environmental conditions via diversification of agricultural production from intensive rice production in order to improve food security (Nguyen et al., 2020b). Simultaneously, sluice gates were constructed as control mechanisms for saline intrusion. Despite these efforts, dry season saline intrusion and drought has continued to cause significant production losses in parts of the lower delta making rice unsuitable for growth during this period (Loc et al., 2021). Local authorities have advised farmers to change crop systems, shifting from three to two rice crops per year, and combine a rotation of alternative crops during the dry season to suit the changing conditions and improve land use efficiency (Le Xuan Dinh et al., 2016). Dry season conditions mean alternative crops could be exposed to saline irrigation water exceeding 4 g L^{-1} which is the trigger for sluice gate closure (Loc et al., 2021) however these concentrations are often exceeded with reports of 10 g L^{-1} occurring 70 km deep into the network of canals in the 2020 drought (MARD, 2020). Concentrations exceeding 15 g L^{-1} have been consistently recorded in locations closer to the ocean (Eslami et al., 2019). The dry season experiences only 10% of the yearly rainfall and the increased occurrence of drought and changes to upstream river flows mean alternative crops may also experience water scarcity stress in addition to salinity (Dang et al., 2020). Therefore, there is an urgent need to find upland crop species displaying high salt tolerance and water use efficiency to cope with the changing climate in the MRD.

Quinoa (*Chenopodium quinoa Willd*.) is a pseudocereal that originated from the Andean regions of South America and since domestication has undergone many morphological changes (Adolf et al., 2013). It is cultivated for its seeds with the grain of quinoa providing a highly nutritional human food source (Angeli et al., 2020). Quinoa can tolerate a range of adverse abiotic growing conditions allowing it to be grown across many geographical regions (Bazile, 2023).

Quinoa has a positive yield response to nitrogen application with rates of 120 kg N/ha increasing grain yield by up to 94% compared to plants not fertilised with nitrogen (Schulte auf'm Erley et al., 2005). Nitrogen application will increase panicle number, plant height and seed weight (AbdElgalil et al., 2023), however excess nitrogen can cause lodging and delayed maturity (Wang et al., 2014). Plant sowing density can influence lodging and affect plant light interception, nutrient competition, and canopy structure, with optimal plant density levels varying by genotype and environment (Minh et al., 2021). High density may increase land-use efficiency but reduce plant biomass and seed size, while low density boosts per-plant yield but lowers total productivity. A Northern Vietnam study by Minh et al. (2021) identified an optimal sowing density of 8 plants/m^2 under the conditions tested; however, these environments did not experience the abiotic salinity stresses characteristic of the Southern Vietnamese Mekong River Delta (MRD).

Quinoa is not currently cultivated in the MRD and remains an uncommon crop in Vietnam, yet its halophytic characteristics (Hussain et al., 2020) and low water requirements (Telahigue et al., 2017) suggest strong potential as an alternative dry-season crop for MRD farmers (Kaveney et al., 2023). Recent research conducted under the cooler growing conditions of northern and central Vietnam has examined quinoa's agronomic performance, including responses to nutrition (Hoang et al., 2021, Nguyen and Chuyen, 2023), plant density (Minh et al., 2021), soil type (Nguyen and Văn Minh, 2022, Văn Minh et al., 2022) and salinity (Long 2016). However, no studies have evaluated quinoa varieties specifically suited to the saline and drought-affected soils of the MRD, where environmental conditions differ substantially from those of northern and central regions. Consequently, both varietal screening and the optimization of agronomic management practices are required to support successful quinoa production in the MRD. Therefore, the aim of this research is to determine suitable quinoa varieties that can tolerate salinity and water limited conditions and optimise their growth through nitrogen fertiliser and sowing density in order to meet the requirements of farming systems affected by climate change.

2. Methodology

Experiments were conducted to investigate the suitability of quinoa for growth in the MRD where varieties were first screened for salinity and irrigation tolerance in a greenhouse pot trial, before selected varieties were tested in two field trials exploring different nitrogen fertiliser rates and sowing densities.

2.1 Salinity and irrigation screening pot trial

The soil used for the pot trial was collected from the 0-20 cm topsoil layer of a rice field in Long An Province, Vietnam. The soil was air dried, sieved (<10 mm), and packed into pots measuring 22 diameter x 25 cm, with each pot containing approximately 9 kg of soil to give a 20 cm soil depth. Soil properties are reported in Table 1.

***Table 1**: Soil properties for the soil used in the glasshouse trial*

Soil property	Unit	Measurement
Soil pH (1:5 water)		4.18
Organic Carbon	%	2.41
Total N	%	0.16
Total P	%	0.03
Available P	mg kg^{-1}	32.00
Al_exchangeable	cmol(+) kg^{-1}	2.73
Ca_ exchangeable	cmol(+) kg^{-1}	4.41
Mg_ exchangeable	cmol(+) kg^{-1}	7.04
K_ exchangeable	cmol(+) kg^{-1}	0.69
Na_ exchangeable	cmol(+) kg^{-1}	1.15
EC (1:5)	dS m^{-1}	0.26

The randomised block pot experiment with 4 replications was designed to screen five quinoa varieties (2-Want, Sluga, Titicaca, 42-Test, and Atlas) under four different salinity and two soil moisture conditions. Ten seeds of each variety were sown per pot at a depth of approximately 1.5 cm in bare soil without mulch. Two weeks after sowing seedlings were thinned to two plants per pot. All pots were watered with tap water (0 g/L NaCl) for the first two weeks before salinity and irrigation treatments commenced. Saline water was introduced starting from the third week after sowing, simulating the point when irrigation with salinised canal water would occur in the Soc Trang region (Ratering Arntz, 2018). Four salinity concentrations were applied (0, 2, 4, and 6 g/L NaCl) by adding water with incremental increases each week of 0.5 g/L until the designated application concentration was reached, after which pots were irrigated with solutions of that concentration as needed. The 0 g/L treatment was irrigated with deionised water throughout the experiment.

Two soil moisture conditions (-22 kPa and -50 kPa) were implemented using Chameleon soil moisture sensors placed at a depth of 10-15 cm to trigger irrigation events. The Chameleon sensors indicate soil tension so readings are independent of soil type (Stirzaker et al., 2017). Chameleons emit a (indicating a moisture content of -20 kPa) or red (indicating a moisture content of -50 kPa), water was applied until the light turned blue (0 kPa).

A basal fertilizer, granular diammonium phosphate (DAP), was applied to all pots at sowing at a rate equivalent to 60 kg/ha. The experiment was conducted under greenhouse conditions at the Institute of Agricultural Sciences for Southern Vietnam from December 2022 to March 2023.

Harvest occurred at maturity, with Titicaca harvested at 75 days after sowing (DAS), and the remaining varieties harvested at approximately 80 DAS. Harvest was carried out intermittently, once every 1–2 days, and completed within 10 days. Aboveground plant material was removed for biomass measurements and air dried prior to recording dry mass of stem and leaves. Roots were washed from the soil, dried until a constant weight was achieved, and then weighed. Individual yield was determined as the dry weight of harvested seeds per plant.

2.2 The effect of nitrogen fertiliser rate in the field

A field experiment was implemented on seasonally saline-affected rice land in Lieu Tu Commune, Tran De District, Soc Trang Province (9°29’13.02”N, 106°6’28.39”E) in the 2022/23 dry season. The soil properties of the site are shown in Table 2.

Table 2: *Soil properties for the Soc Trang field site.*

Soil property	**Unit**	**Measurement**
Soil pH (1:5 water)		5.21
Organic Carbon	%	4.57
Total N	%	0.34
Total P	%	0.04
Available P	mg kg^{-1}	10.70
Al_exchangeable	cmol(+) kg^{-1}	0.29
Ca_ exchangeable	cmol(+) kg^{-1}	7.43
Mg_ exchangeable	cmol(+) kg^{-1}	7.10
K_ exchangeable	cmol(+) kg^{-1}	0.71
Na_ exchangeable	cmol(+) kg^{-1}	3.18
Electrical conductivity EC (1:5)	dS m^{-1}	0.40

A randomised block experiment of two factors, quinoa variety and urea nitrogen fertiliser rate, was applied to 4 replicates. The two best performing varieties from the pot trial screened for salinity tolerance (Section 2.1) were selected for field testing: 2-Want and Titicaca. Four application rates of urea were applied (0, 40, 80 and 120 kg N/ha) in even split applications occurring 10, 25 and 40 DAS. Super phosphate (90 kg P/ha) and potassium chloride (75 kg K/ha) were used as a source of phosphorus and potassium fertilizer and were applied to all treatments at sowing. Plots 4 x 5 m were formed by creating three raised beds of 0.9 m width, and 30 cm high. Bunds of earth (0.2 m high and 0.3 m wide) separated the plots to ensure soluble nutrients could not move from each treated plot.

Quinoa varieties were direct sown at a density of 8 plants/m^2. Each raised bed had three rows (30 cm apart) with 25cm between plants. All beds were mulched with rice straw at 1kg/m. Sowing commenced at the end of the rainy season on the 31/10/22.

Plots were irrigated with canal water in irrigation events triggered when the Chameleon soil moisture sensor lights indicated red (<50 kPa) and occurred until the sensor light turned blue (>-22 kPa). The irrigation cycle depended on weather conditions. All plots were irrigated simultaneously. Soil moisture was monitored at a depth of 0–15 cm, with the Chameleon sensor installed at approximately 15 cm depth. Rainfall, humidity and temperature were recorded hourly using a weather station.

Plants were harvested manually on a single day (14/01/2023). Dry biomass was collected from the stems, leaves, and roots (after being washed and dried until a constant weight was achieved) and then weighed. Individual yield was determined as the dry weight of harvested seeds per plant.

2.3 The effect of sowing density in the field

A second field trial was conducted in the 2023/2024 dry season on the same site as the 2022/23 fertiliser field trial following two subsequent rice crops. The nitrogen rate field trial (Section 2.2) demonstrated Titicaca showed promising characteristics for growth in the MRD and thus was chosen for further investigation in a plant sowing density trial as means to decrease lodging. Densities of 12.5,25 and 50 plants/m^2 were chosen based on previous studies by Taaime et al. (2023) and Nguyen Van Minh et al. (2020). Three sowing dates were also investigated in an early (February 27, 2024) mid (March 12, 2024) and late (March 26, 2024) sowing based off the previous rice harvest schedule.

Each experimental plot had an area of 12 m^2 (4m x 3m). Drainage ditches were placed between the plots. Phosphorus (super phosphate) was applied to all plots at a rate of 90 kg P_2O_5/ha. Nitrogen (urea) and potassium (potassium chloride, KCl) were applied in three separate applications (Table 3). Superphosphate (90 kg P_2O_5/ha) and Potassium Chloride (90 kg K_2O/ha) were uniformly applied across all treatments. Urea was applied at a rate of 0.64 g/plant and varied based on sowing density.

Table 3: *Fertiliser type, rate and split application for the sowing density field trial at Soc Trang.*

Fertilizer type	**Rate (Kg/ha)**	**1st addition (10 DAS)**	**2nd addition (25 DAS)**	**3rd addition (40 DAS)**	**4th addition (50 DAS)**
Super phosphate (16% P_2O_5)	90 P_2O_5	100%			

Urea (46%N)	120 N	15%	40%	30%	15%
KCl (60% K_2O)	90 K_2O	15%	25%	35%	25%

Dry biomass was collected on 3/6/2024 from the stems, leaves, and roots (after being washed and dried until a constant weight was achieved) and then weighed. Individual yield was determined as the dry weight of harvested seeds per plant. The lodging rate was determined for each treatment plot based on the number of lodged plants per plot.

2.4 Statistical analysis

Statistical analyses were performed using Genstat version 22 with two-way ANOVA of salinity and variety, and irrigation and variety for yield, biomass, root mass, and water use efficiency (WUE). Genstat was applied to the experimental content of experiment 1 (section 2.1) and experiment 2 (section 2.2). Minitab (Minitab, LLC, Pennsylvania, US) was used for one-way ANOVAs of sowing density, examining biomass, root mass, and lodging rate. Minitab was applied to the content of experiment 3 (section 2.3).

Results

3.1 Salinity and irrigation screening pot trial

Salinity

Atlas had the greatest shoot and root biomass but was significantly influenced by the presence of salinity (Figure 1 A and B). Titicaca had the smallest shoot biomass compared to all other varieties. It also had the smallest root mass but was comparable to saline affected Sluga and 42-test varieties. The grain yield of all varieties was significantly affected by the presence of increasing salinity. Again, the low biomass production of Titicaca was associated with significantly higher yield than any other variety for the same salinity. Though the yield of Titicaca was sensitive to salinity (Figure 1C), the yield at the highest salinity (6 g/L) was greater than all other varieties with the exception of 2-want, Sluga and 42-test in the absence of salinity. The WUE of varieties decreased with increasing salinity (Figure 1D). Titicaca resulted in the greatest WUE in the presence of salinity.

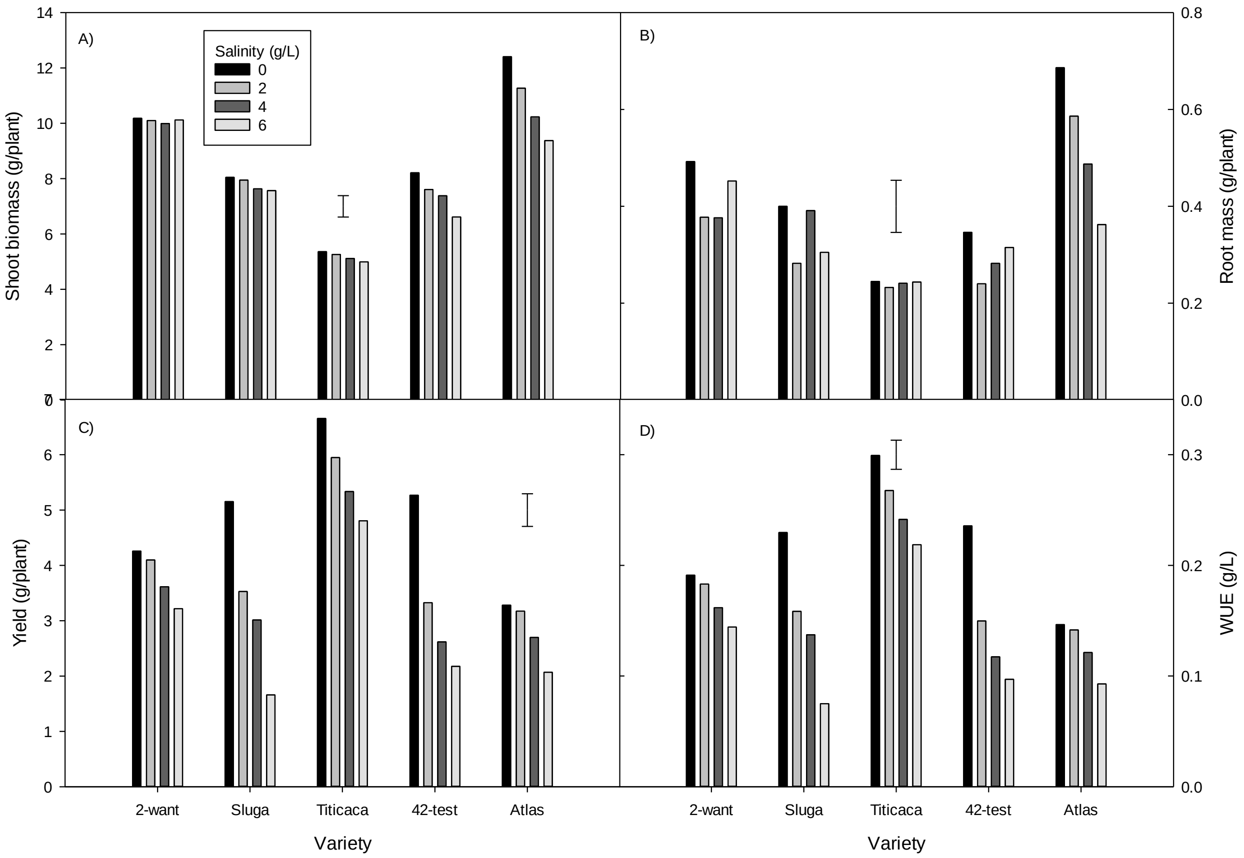

***Figure 1**: The shoot biomass (g/plant), root mas (g/plant), yield (g/plant) of five quinoa varieties grown in increasing concentrations of salinity (0, 2, 4 and 6 g/L NaCl). LSD bar indicates significant interaction between salinity concentration and quinoa variety for shoot biomass, root mass, yield and water use efficiency (p=0.05).*

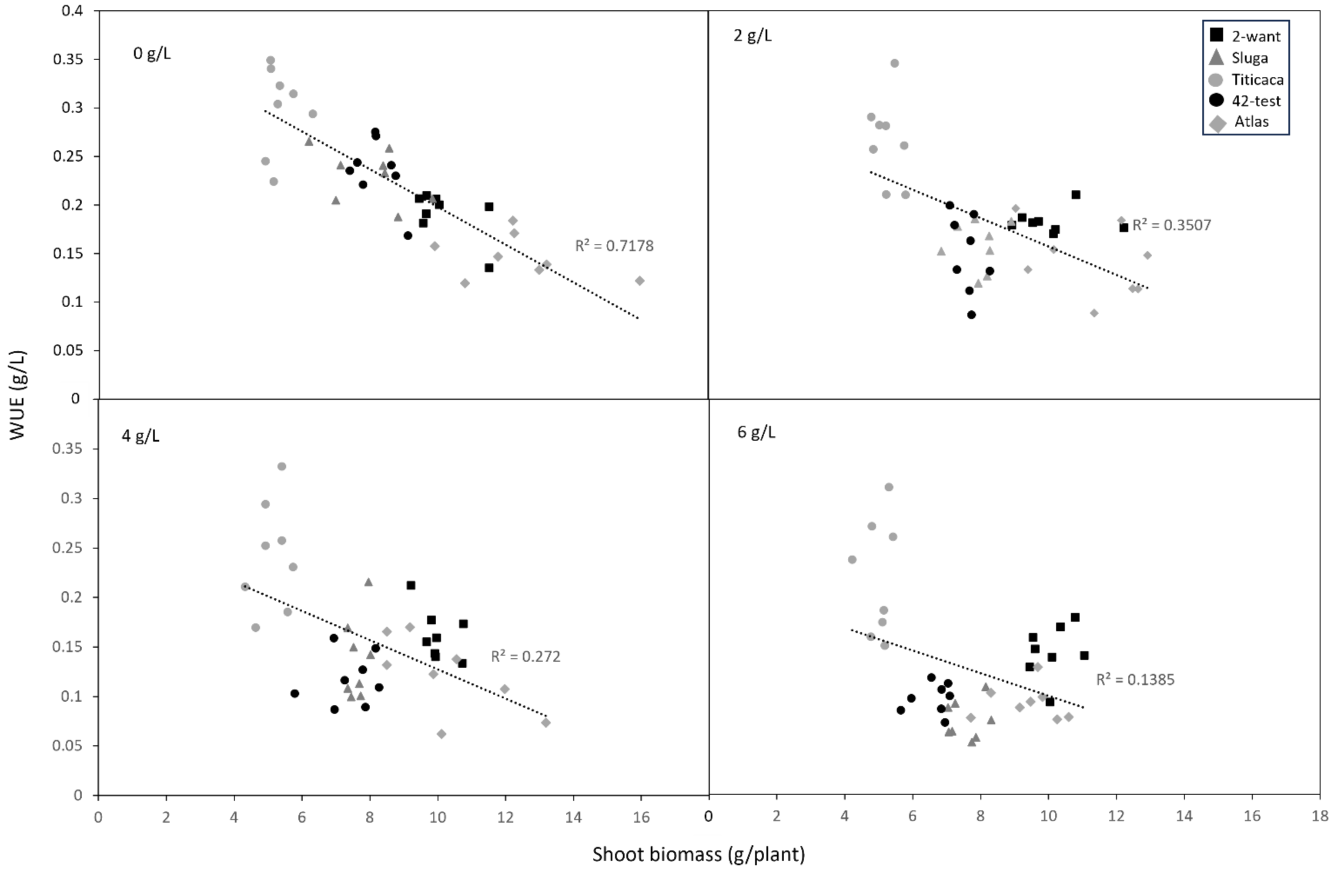

Figure 2: *The negative correlation between water use efficiency (WUE (g/L)) and shoot biomass (g/plant) for the five quinoa varieties screened for saline tolerance (2-want, Sluga, Titicaca, 42-test and Atlas) at the four salt concentrations investigated of 0, 2, 4 and 6 g/L NaCl. The linear correlation coefficient is calculated on the average of all five quinoa varieties and displayed as a dashed line.*

A strong negative correlation between WUE and shoot biomass existed in non-saline conditions (Figure 2). As plant biomass increased, its WUE decreased with varieties including Atlas and 2-want demonstrating the lowest WUE. As salt concentrations increased, the correlation coefficient became weaker with biomass impacting WUE the least at 6 g/L.

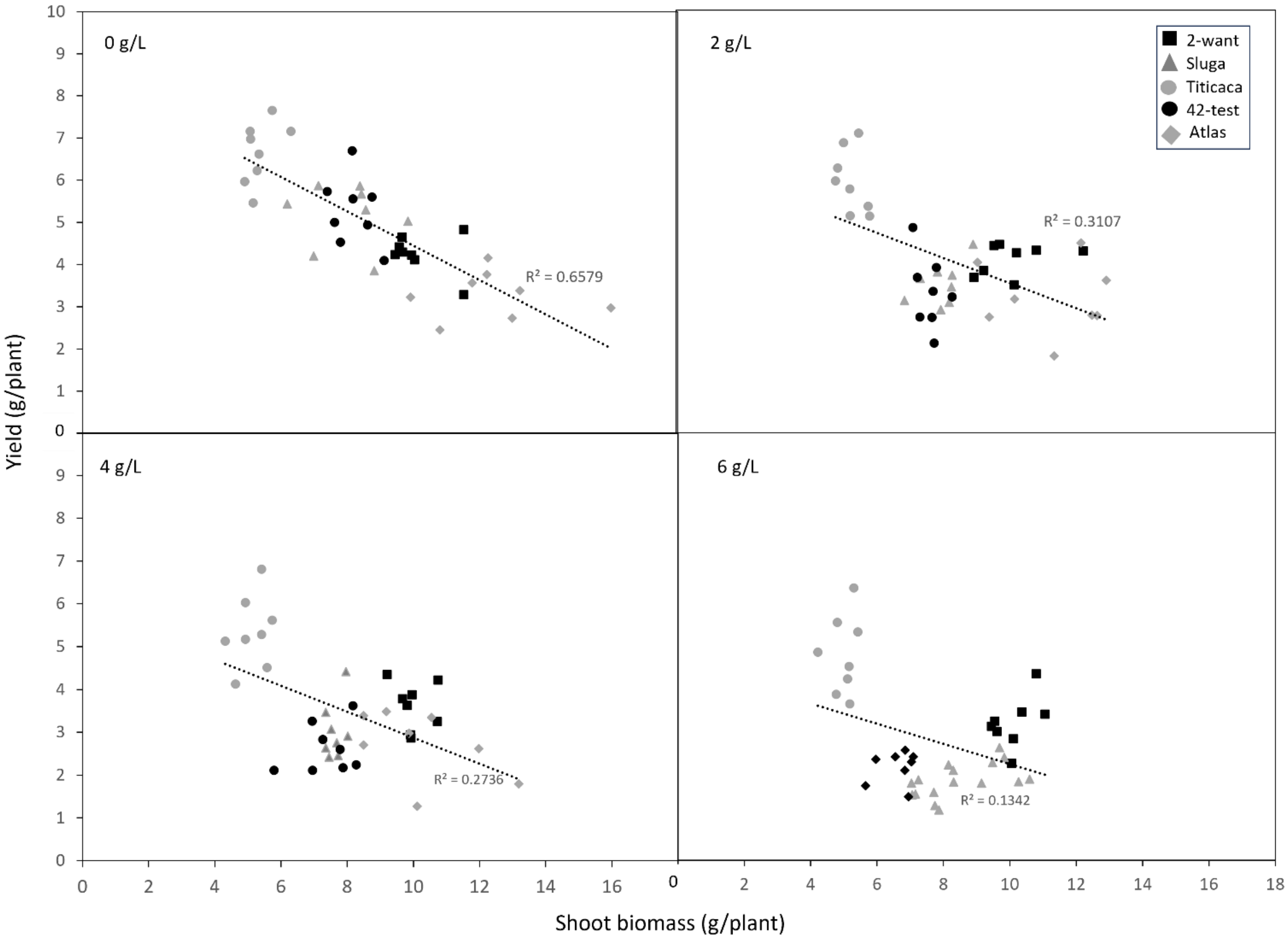

***Figure 3**: The negative correlation between yield (g/plant) and shoot biomass (g/plant) for the five quinoa varieties screened for saline tolerance (2-want, Sluga, Titicaca, 42-test and Atlas) at the four salt concentrations investigated of 0, 2, 4 and 6 g/L NaCl. The linear correlation coefficient is calculated on the average of all five quinoa varieties and displayed as a dashed line.*

A negative correlation between yield and shoot biomass existed across all salt concentrations but was strongest at the lowest salt concentrations (Figure 3). As salt concentrations increased, the correlation strength became weaker. Yield implications occurred for plants like Atlas that had a higher biomass, whilst Titicaca was a variety that had low shoot biomass and higher yields.

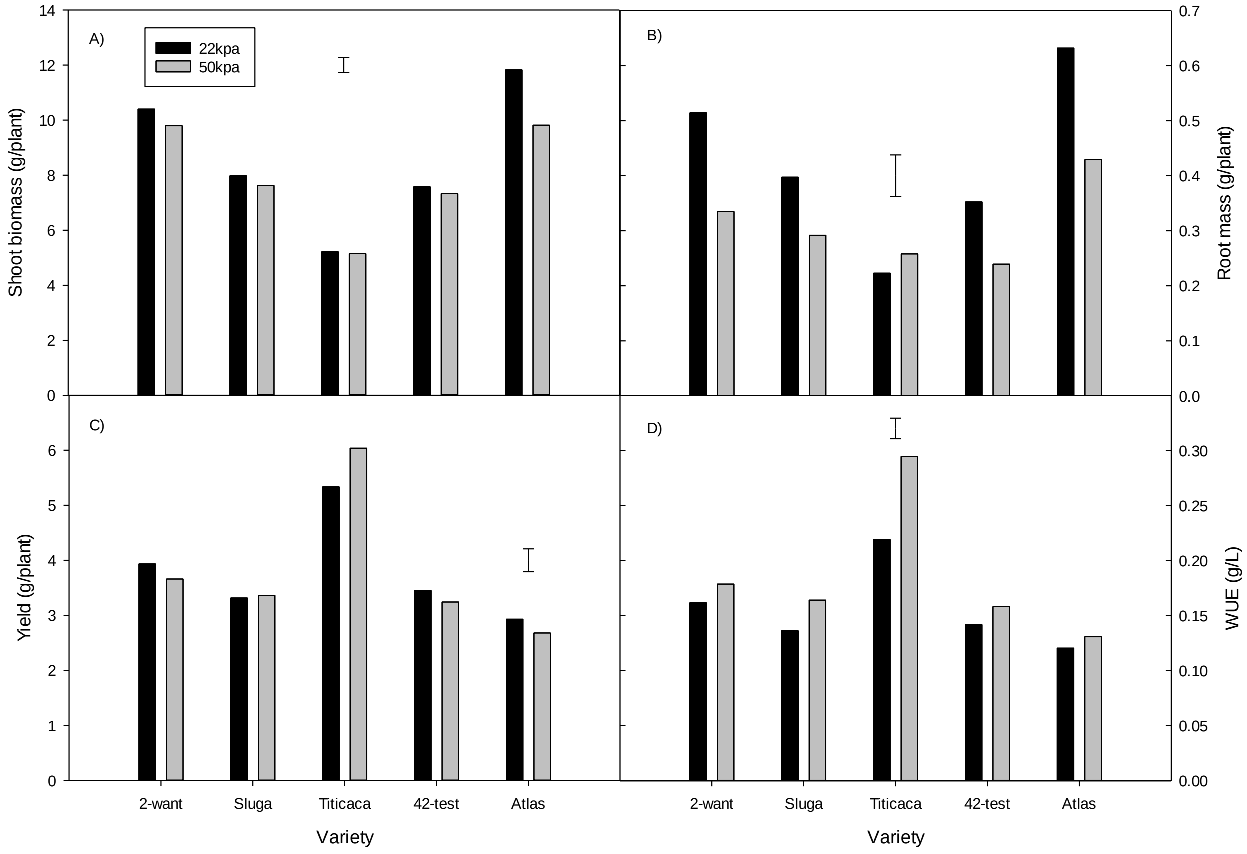

Figure 4: *The shoot biomass (g/plant), root mas (g/plant), yield (g/plant) of five quinoa varieties grown with two different irrigation schedules (22 kPa and 50 kPa). LSD bar indicates significant interaction between irrigation and quinoa variety for shoot biomass, root mass, yield and water use efficiency (p=0.05).*

Irrigation

There were no significant main effects due to irrigation for any measured parameter. Significant interactions of irrigation treatment and variety existed for plant biomass and yield (Figure 3). The varieties that had more shoot biomass also had more root biomass (2-want and Atlas) with irrigation at 22 kPa having significantly higher shoot and root mass for these treatments (Figure 3 A and B). Root mass was also greater at 22 kPa for Sluga and 42-test, though there were no differences in the shoot biomass of those variety due to irrigation. Interestingly, Titicaca had the lowest above and below ground biomass but was unaffected by irrigation. Titicaca returned the highest grain yield and 50 kPa was significantly greater than 22 kPa (Figure 3C). This resulted in Titicaca having the greatest WUE and 50 kPa being significantly better than 22 kPa for Titicaca (Figure 3D).

3.2 The effect of fertiliser rates in the field

Yield

There was a clear difference in yield between the two quinoa varieties under field conditions (Figure 4). Titicaca demonstrated the potential for achieving harvestable yields when grown in MRD dry season conditions. Increasing the nitrogen fertilizer application rate significantly contributed to higher yields with 120 kg N/ha resulting in the highest yield. In contrast, the 2-Want variety did not produce measurable yields under the same planting time and experimental conditions.

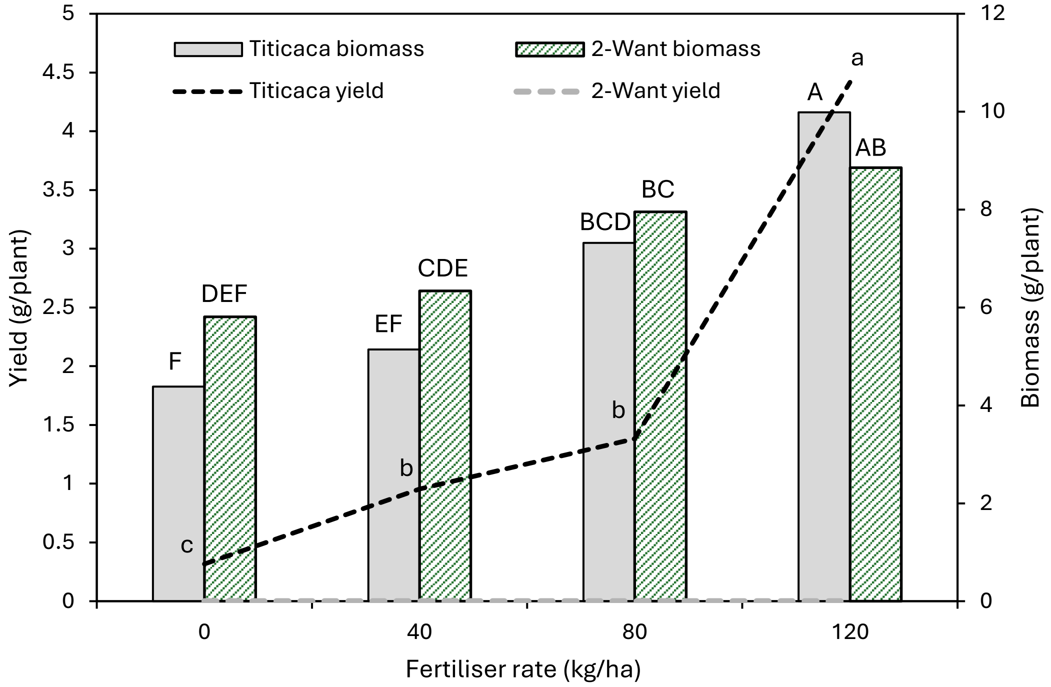

Figure 5: *The effects of increasing fertiliser rates on the biomass (columns) and yield (line) of the quinoa varieties 'Titicaca' and '2-Want'. Different letters indicate significant difference between treatments (capital letters for biomass and lowercase for yield) and variety (P<0.05).*

Biomass

The results showed that the addition of nitrogen fertilizer positively impacted the biomass of both varieties (Figure 4). As nitrogen concentration increased, plant biomass also increased. In the treatment without nitrogen supplementation, biomass was significantly lower compared to the other treatments for both varieties. Despite 2-Want not producing harvestable yield, this varieties biomass was the same as Titicaca for all nitrogen rates.

3.3 The effect of sowing density in the field

Yield and biomass

Titicaca plant shoot biomass decreased as sowing density increased (Table 4), although this was not significant. A sowing density of 12.5 plants/m^2 resulted in the highest biomass compared to the other two densities (25 plants/m^2 and 50 plants/m^2). At harvest, there was an occurrence of shrivelled seeds and failure in seed formation which resulted in negligible seed yield. The early sowing date was the only successful trial where plants reached maturity and produced harvestable yield. Plants from the mid and late sowing dates failed to grow and produce seeds.

Table 4: *The effect of sowing density on fresh and dry root mass, and fresh and dry biomass of Titicaca in field conditions. Average and standard deviation are reported.*

Density rates (plants m^2)	**Fresh shoot biomass** (g plant^{-1})	**Dry shoot biomass** (g plant^{-1})	**Fresh root mass** (g plant^{-1})	**Dry root mass** (g plant^{-1})
12.5	11.15 ± 4.48	2.02 ± 0.81	1.81 ± 1.12/	0.54 ± 0.34
25	7.00 ± 1.81	1.47 ± 0.34	2.74 ± 0.13	0.80 ± 0.04
50	5.13 ± 5.46	1.05 ± 1.09	3.57 ± 1.27	1.10 ± 0.39
	NS	NS	NS	NS

Root mass

The results indicated an increase in root biomass with higher planting densities, although this was not significant (Table 3). A planting density of 50 plants m^2 produced the highest root biomass compared to the other two densities (12.5 plants/m^2 and 25 plants/m^2).

Lodging rate

Sowing density had no significant impact on quinoa lodging rate (Figure 6). A trend of higher planting densities (25 and 50 plants/m^2) having increased lodging rates existed, but it was not statistically different.

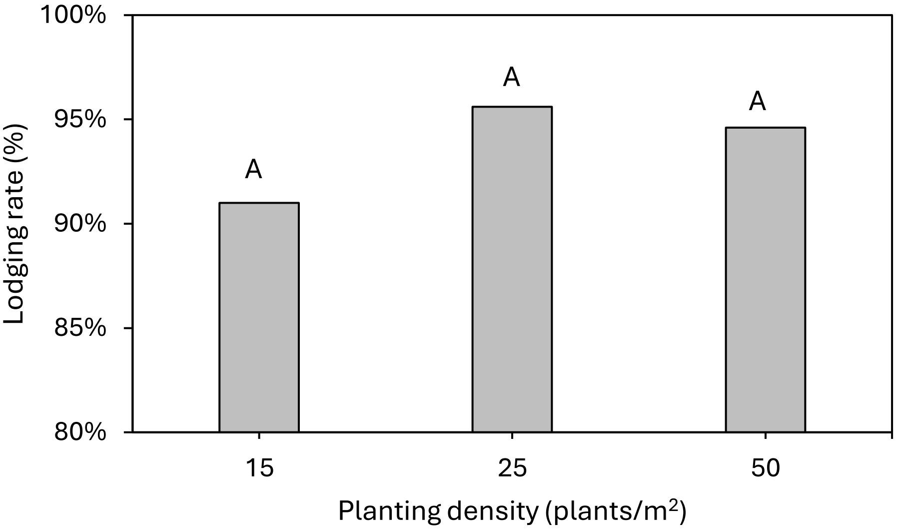

Figure 6*: The effect of planting density on lodging rate of Titicaca in field conditions. Columns sharing the same letter are not statistically significant.*

Temperature during the field trial

During the flowering stage, a heat wave occurred from the beginning of April that resulted in daytime temperatures consistently exceeding 34°C and nighttime temperatures above 25°C (Figure 7). The heat wave period where daily temperatures exceeded 34°C lasted for 21 days. This was followed by patterns of peaks and troughs where daytime temperatures reached 34-36℃ and dipped to 31℃ every 6-7 days. The pattern of these temperatures was considered atypical for the MRD dry season.

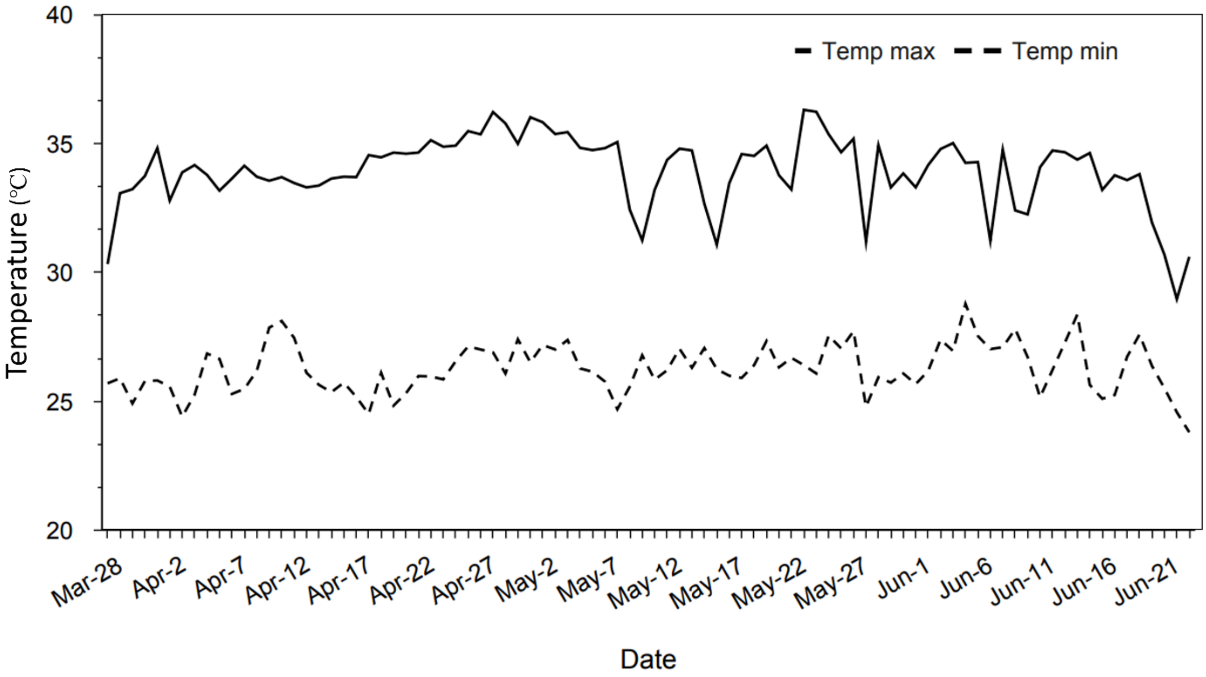

Figure 7: The maximum (Temp max) and minimum (Temp min) air temperatures in degrees Celsius and rainfall for the 2024 sowing density field trial at Lieu Tu Commune, Tran De District, Soc Trang Province Vietnam.

4. Discussion

4.1 The importance of variety selection to suit MRD conditions.

Saline intrusion (Loc et al., 2021), droughts (Lee et al., 2019) and water scarcity (Anh et al., 2019) are prominent dry season constraints to production in the MRD. Selecting quinoa varieties that can tolerate these conditions will increase their likelihood of success as an alternative crop to rice. Quinoa varieties have been screened for salinity tolerance before (Adolf et al., 2012, Cai and Gao, 2020, Hussain et al., 2020), including in temperate northern Vietnam (Long, 2016, Nguyen Viet, 2016). However, quinoa has not been evaluated under the tropical, saline and drought affected conditions characteristic of southern Vietnam, highlighting a critical research gap. The greenhouse results showed increasing salt concentrations negatively affected the yield of all quinoa varieties, though the degree of salinity tolerance was variety dependent (Figure 1). Lowest yielding varieties (Atlas and 42-test) had the greatest biomass (Figure 1A) whilst Titicaca produced the highest yield (Figure 1C) across all salt concentrations and recorded the lowest plant biomass. The negative correlation between yield and shoot biomass (Figure 3) may be due to transpiration rates. In saline conditions, quinoa plants reduce transpiration rates by decreasing stomatal density and leaf area (Turcios et al., 2021). Varieties more efficient at reducing leaf area and consequently biomass, save water by transpiring at lower rates and display a greater salinity tolerance (Agirresarobe et al., 2022). Additionally, Titicaca recorded the lowest root weight compared to other varieties (Figure 1B).

Decreased root growth in saline tolerant varieties reduces the excessive uptake of toxic Na^+ and Cl^- (Cai and Gao, 2020).

High water use efficiency (WUE) is a desirable trait when assessing the suitability of varieties for development in the MRD. In conditions of water scarcity (Anh et al., 2019), crop varieties that can achieve high yields with less irrigation water are more suitable, especially during the dry season. In this experiment, Titicaca had significantly higher yield and WUE at both -22 kPa and -50 kPa than any other variety, and the lowest root and biomass (Figure 4 B&D). The variety *Atlas* that yielded significantly less than Titicaca, recorded much higher root mass and lower WUE (Figure 2 B&D). It was also found through the correlation that WUE improved with lower biomass varieties (Figure 2). Drought tolerant quinoa varieties will decrease root diameter in order to increase root length and promote water uptake (Nguyen et al., 2021). In the present study, Titicaca was the only variety to have a significant yield difference between irrigation treatments, with drier conditions interestingly promoting higher yields. Irrigating quinoa using alternative root-zone drying techniques actually improved quinoa plant growth and WUE compared to full and deficit irrigation (Yang et al., 2016). The varieties Atlas and 2-Want have previously exhibited drought tolerant phenotypes when grown in Northen Vietnam (Nguyen et al., 2021), and thus the difference between -22 kPa and -50 kPa in the current study may not have been enough to induce drought like conditions. Decreased yields may be attributed to experimental factors other than drought.

Whilst salinity tolerance and WUE is an important trait for successful quinoa growth in the MRD, the influence of other abiotic conditions including temperature was evident through the performance of Atlas and 2-Want (Figure 1). Both varieties have shown promising yield potential under saline conditions in Northern Vietnamese trials (Long, 2016, Nguyen et al., 2020a, Hoang et al., 2021). However, Atlas performed poorly in MRD greenhouse conditions (Figure 1) and 2-Want did not produce any harvestable yield in the field (Figure 5). High temperatures, particularly during flowering and seed formation, are among the most limiting factors for quinoa yield (Yang et al., 2016, Hinojosa et al., 2019). Temperatures in the MRD dry season remain relatively constant with daytime temperatures ranging between 27-34℃ and nighttime between 23-27℃ (Kontgis et al., 2019), yet these temperatures are signfincantly higher than the temperate conditions of Northern Vietnam. Additionally, the MRD is experiencing increased occurrences of drought and heat waves (Phan et al., 2020, Nghia et al., 2024), like the heat wave that occurred towards the end of the 2024 sowing density field trial where negligible yield was harvested (Table 6). This same drought period resulted in over 70,000 MRD households experiencing domestic water shortages (UNICEF, 2024). The MRD is highly vulnerable to climate change (WorldBank, 2021) and quinoa is sensitive to high temperatures (Hinojosa et al., 2019). Whilst growth rate promotion occurs when temperatures increase from 18/8 ℃ (day/night) to 25/20 ℃ (Yang et al., 2016), temperatures exceeding 33°C have negative effects on crop yield (Alvar-Beltrán et al., 2019). High temperatures exceeding 40/24°C can reduce the viability of quinoa pollen grains by 30% to 70%, leading to decreased pollination rates and seed formation (Hinojosa et al., 2019). Temperatures exceeding 35°C can have significant negative effects on crop yield (Peterson and Murphy, 2015, Walters et al., 2016),. The occurrence of empty seeds or non-formation of seeds, leading to a reduction or complete loss of seed yield, is likely due to the combination of low relative humidity (below 30%) and high temperatures (35°C). As temperatures rise, quinoa flower clusters may fail to produce seeds or may contain empty seed casings. Nighttime temperatures of 20–22°C (about 4°C higher than the surrounding air temperature) during flowering can also reduce yield by 23–31% (Lesjak and Calderini, 2017). In the 2024 field trial, day and night temperatures exceeded 34℃ and 25℃ respectively (Figure 7) for up to 21 consecutive days. This prolonged heat period occurred during anthesis and likely resulted in sterilisation and consequential grain failure (Table 4), as experienced by other trials with high temperatures (Taaime et al., 2023). The combined effects of salinity and temperature on quinoa yield have been investigated with varying results. Negligible effects were found when maximum temperatures only reached 28 °C (Becker et al., 2017), however the combined effects of salinity, drought and high temperature (38 ℃) caused up to a 50% yield decline (Abbas et al., 2023). Interestingly, of the four varieties examined, Titicaca demonstrated greater tolerance to temperature, salinity and drought stresses than other varieties. In the present study, combined salinity and high MRD dry season temperatures in the greenhouse likely contributed to yield reductions in less tolerant varieties (Sluga, 42-Test and Atlas), and complete yield loss in the moderate temperature field conditions of 2023 (2-Want). Ensuring that the reproductive phases of Titicaca avoid the peak in dry season temperature may contribute to this variety's success in the MRD.

4.2 Optimising quinoa growth in the MRD

Quinoas adaptability to adverse conditions and tolerance of abiotic stresses demonstrate potential as an alternative crop in the MRD dry season, however understanding its growing requirements could provide farmers with greater yield potentials and decrease the risk of crop failure. The addition of nitrogen fertiliser to Titicaca at low rates (40 kg/ha) improved yields by 200%, and moderate rates (80 kg/ha) by over 300% (Figure 5). The further addition of an extra 40 kg/ha (120 kg/ha) signfincantly increased yield by over 1200% from the control. Increasing fertiliser also increased the biomass of '2-Want' but no grain was harvested, likely due to this varieties susceptibility of temperature stress causing sterilisation at anthesis (Hinojosa et al., 2019). Quinoa yield responses to N fertiliser have been explored before in water-limited environments (Taaime et al., 2023) and saline conditions (Hoang et al., 2021) with varying results. Under semi-arid rainfall limited conditions, 40 kg N/ha achieved the greatest nitrogen use efficiency (Taaime et al., 2023), whilst the optimum N rate in saline conditions was found to be 90 kg N/ha with yield decreasing at rates of 150 kg N/ha (Hoang et al., 2021). In the current study, yield response was still linear (Figure 5) suggesting that the fertiliser rates examined of 120 kg N/ha may be limiting yield potential in MRD conditions. Other studies examining quinoa growth in conditions without abiotic pressures found early plant vigour and biomass gains at rates over 75 kg N/ha improved all growth and yield traits (Basra et al., 2014) whilst quinoa grown under mediterranean conditions yielded highest at 150 kg N/ha (Geren, 2015). To produce 1 ton of quinoa grain yield it is suggested that 60 kg N is required (Taaime et al., 2023). Nitrogen rates do not affect the growth duration of quinoa (Hoang et al., 2021) and thus changes to planting date may be required to limit abiotic stresses during sensitive growth stages including anthesis.

Saline intrusion, water scarcity and temperatures peak towards the end of the MRD dry season during April and May (Eslami et al., 2021, Phan et al., 2020). Quinoa was planted in the field in late February and March 2024 with anthesis occurring in the period of peak abiotic stresses. Aforementioned, temperature stress during anthesis likely caused grain sterility (Hinojosa et al., 2019) in 2-Want (Figure 5) and Titicaca (Table 4). Manipulating the cropping calendar to sow quinoa earlier in the dry season may reduce the risk of abiotic stresses impacting yield. Quinoas susceptibility to waterlogging (González et al., 2009) may present alternative issues to seedling establishment if sown too soon after the second rice crop, yet management techniques like raised beds may mitigate these risks (Bakker et al., 2010).

Plant lodging occurred at end of the growth period for the first field trial (2023) that assessed the effects of N fertiliser on quinoa yield (Figure 5). Exploring plant density in the 2024 field trial found that sowing rates had no effect on lodging percentages (Figure 6). In the relatively cooler rain-fed climate (temperature ~25 °C) of Vietnam's Central Highlands, an optimal planting density of 80,000 plants/ ha was found to maximise biomass and yield (Minh et al., 2021). Other trials also based in the central highlands of Vietnam have utilised a range of 66,000 plants/ha to 133,333 (Nguyen and Chuyen, 2023, Nguyen and Văn Minh, 2022). Quinoa is susceptible to stem lodging caused by wind force, with a significant increase in lodging percentage occurring as plant density increases (Wang et al., 2021). However, the trade-off between yield and lodging means that a reduction in sowing density from 400,000 plants/ha to 200,000 plants/ha signfincantly reduced quinoa yield. Increased lodging also occurs in well irrigated soil with less negative soil matric potentials promoting plant height and consequently increasing their susceptibility to lodging. In the present study, sowing rate did not signfincantly affect biomass or root mass (Table 4) and yield effects could not be measured do to temperature stresses. Selecting varieties with decreased plant height and thicker stems may reduce lodging occurrence (Tang et al., 2024). Further studies investigating plant height and sowing density are still required for the optimisation of quinoa growth in the MRD.

Conclusion

Given the increasingly complex climate change situation in the Mekong River Delta, crop diversification is crucial for future adaptation and development. Growing quinoa in saline and water limited areas of the MRD requires a variety that can tolerate these conditions and produce harvestable yield. Titicaca displayed the greatest saline tolerance through increased yield despite being exposed to 6 g/L salt concentration and -50 kPa soil moisture irrigation conditions. However, field results indicate significant challenges in growing quinoa in real-world conditions with high temperatures providing yield challenges in the MRD. Plant yield increased with nitrogen application however lodging still occurred at a range of sowing densities. Further research is required to identify suitable technical solutions, including manipulating the cropping calendar to reduce abiotic stresses at anthesis. Timing sensitive reproductive phases to avoid peaks in temperature, varieties like Titicaca could provide a suitable alternative crop to rice for growth in the MRD dry season.

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Paper 2: Exploring the suitability of cowpea as an alternative dry season crop to rice in the Mekong River Delta, Vietnam

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Abstract

Cowpea (*Vigna unguiculata* L. Walp.) is a drought-tolerant legume with potential to diversify cropping systems in the saline- and water-limited environments of the Mekong River Delta (MRD). However, its growth and responses to irrigation and phosphorus (P) fertilizer management under these conditions have not been explored. Field trials were conducted over the 2023 and 2024 dry seasons to evaluate the effects of irrigation regime and P application on growth, yield, physiological stress responses, and soil salinity dynamics in two cowpea varieties (TN142 and TLP69). Irrigation treatments included continuous, intermittent, and farmer-practice irrigation, while P was applied at increasing rates up to 90 kg P_2O_5 ha^{-1}.

Biomass accumulation was not significantly affected by irrigation regime in either variety, reflecting cowpea's inherent tolerance to variable water availability. Yield trends favoured the farmer-practice irrigation, particularly for TN142; however, intermittent irrigation did not result in significant yield penalties, indicating potential for improved water-use efficiency without compromising productivity. Varietal differences in stress response were evident, with TN142 exhibiting significantly higher proline accumulation in shoots and roots than TLP69, despite similar biomass production, suggesting greater osmotic stress sensitivity. In contrast, TLP69 maintained more stable yields across irrigation regimes, indicating superior stress tolerance and water-use efficiency. Phosphorus fertilization significantly increased dry biomass and grain yield across both seasons, with the strongest responses observed at 60–90 kg P_2O_5 ha^{-1}, confirming P availability as a major constraint to cowpea productivity in the MRD. Soil electrical conductivity declined over the growing season despite increasing salinity of irrigation water, likely due to seasonal rainfall and salt leaching.

Overall, the study demonstrates that cowpea can be productively grown in the MRD when supported by appropriate irrigation and fertilizer management. Intermittent irrigation combined with adequate P supply and stress-tolerant, nutrient-efficient varieties offers a promising strategy to enhance water-use efficiency and sustain yields under increasing salinity and water scarcity.

1. Introduction

Dry season saline intrusion and fresh water shortages in the Mekong River Delta (MRD) Vietnam have been exacerbated by both natural (Wang et al., 2021; Loc et al., 2021) and anthropogenic factors (Anh et al., 2019; Binh et al., 2020). The flow of freshwater from the Mekong River decreases during the dry season that spans from November to April, allowing saline water from the South Sea to penetrate deeper into the Delta's freshwater systems. This phenomenon is particularly intense in the southern provinces of the MRD, such as Soc Trang, Long An, Tien Giang, and Ben Tre (Tran et al., 2019). The dry season rice crops are highly vulnerable to salinity, with soil salinization and periods of drought resulting in reduced yields and crop failures (Paik et al., 2020). In response, local governments have recommended the abandonment of dry season rice and adoption of alternative crops to rice suitable for those regions (MARD, 2020).

Cowpea (*Vigna unguiculata*) is a legume of important agricultural value providing a high protein food source for human or livestock consumption. The plant's ability to fix atmospheric nitrogen can also provide important systems benefits through significantly increased soil nitrogen concentrations (Brito et al., 2011) with annual contributions of up to 300 kg N ha^{-1} (Yahaya, 2019) via effective symbiotic relationships with *Rhizobium*. The utilisation of legumes can reduce a rotational cropping system's reliance on nitrogen fertiliser inputs and decrease greenhouse gas emissions (Peoples et al., 2019). Cowpea has historically been grown in marginal production zones due to the plant's resilience to abiotic stresses (Düzdemir et al., 2009; Sansa et al., 2025).

Cowpea is relatively tolerant to soil salinity compared to many other crops. This tolerance is thought to arise from mechanisms such as the exclusion of sodium ions from shoot tissues (Le et al., 2021) and the ability to compartmentalize toxic ions in vacuoles, thereby preventing them from interfering with metabolic processes (Ashraf et al., 2012). Increased proline and soluble protein contents of saline tolerant varieties under salt stress help maintain osmotic balance and cellular turgor pressure (Mini et al., 2019). The application of proline to cowpea plants grown at 150 mM NaCl helped maintain plants K^+/Na^+ ratio, sustained relative water content by 40%, and mitigated oxidative stress by reducing hydrogen peroxide content (Al-Maskari and Yaish, 2025). The importance of variety selection is highlighted in a study where one cowpea genotype exhibited physiology and yield related characteristics at 50 and 100 mM salt concentration whilst other genotypes did not display these same characteristics (Akter et al., 2022).

The drought resistance of cowpea is primarily due to the plant's ability to undergo physiological adjustments, such as stomatal regulation, osmotic adjustment, and leaf folding, which help conserve water and minimize water loss through transpiration (Mishra et al., 2022). Additionally, cowpeas possess the ability to complete their life cycle within a relatively short period, often maturing in less than 90 days (Le et al., 2021), which is beneficial in areas with unpredictable rainfall patterns. Research has shown that different cowpea varieties exhibit varying degrees of drought tolerance, particularly at reproductive phases (Sansa et al., 2025). For instance, varieties that maintained biomass production under drought conditions resulted in higher yields. Drought tolerant varieties possess traits such as better stomatal conductance regulation and higher transpiration efficiency, enabling them to cope more effectively with water stress (Lima et al., 2019).

Whilst cowpea is relatively tolerant of abiotic stresses, its production efficiency can be improved through effective irrigation (Oumarou et al., 2015) and fertilizer management (Bawa, 2020). Targeted irrigation strategies that maximise water use efficiency (WUE) enable farmers to conserve water while maintaining productive yields, particularly under water-limited conditions (Edwin Kimutai et al., 2020). Irrigation approaches such as deficit irrigation, in which water is applied below crop evapotranspiration requirements, can enhance crop water use efficiency by encouraging more efficient utilization of available soil moisture. Chameleon soil moisture sensors provide an accessible and interactive tool application (Kyei-Boahen et al., 2017) which has been shown to mitigate the impacts of abiotic stress in plants (Baroowa et al., 2022). Given the novelty of cowpea production in the MRD, the effects of combined irrigation and phosphorus fertilizer management remain unknown and warrant investigation prior to large-scale adoption. Accordingly, the following field trials aim to assess the suitability of cowpea as an alternative crop and to optimise its growth under MRD farming conditions.

2. Materials and methods

Two separate field experiments were conducted over three consecutive dry seasons (2022–2024) to assess the suitability of cowpea for growth in the MRD. The trials were carried out during the dry season at a field site located in the Lieu Tu commune, Tran De district, Soc Trang province. The experimental site was a rice field affected by both saline and drought stresses, representative of challenging growing conditions commonly experienced in the region during the dry season.

2.1 Sensitivity of cowpea varieties to continuous and intermittent irrigation using saline water in field conditions

The first field trial, which examined the sensitivity of cowpea to irrigation management, was conducted during the 2022 dry season from March to June. The experiment followed a randomized completely block design (RCBD) with four replications. Two cowpea varieties, TN142 and TLP69, were selected for evaluation. Irrigation water was taken from nearby canals, with saline levels gradually increasing over the crop growth period. Three irrigation treatments were applied including farmer's irrigation (T1), constant irrigation (T2), and intermittent irrigation (T3). Both the continuous and intermittent irrigation treatments were scheduled using Chameleon soil moisture sensors (VIA, 2023) installed at a depth of 10–15 cm. Continuous irrigation was applied when the sensor indicated a blue light (0 to −22 kPa), whereas intermittent irrigation was applied when the sensor displayed a red light (> −50 kPa). The control treatment followed local farmers' conventional irrigation practices, in which soil moisture was assessed without instrumentation.

The planting density was 48 plants per m^2. Raised beds approximately 20 cm high and 1–1.2 m wide were prepared for planting. Seeds were sown in two rows per bed, with a spacing of 80 cm between rows and 30 cm between plants within rows. All beds were covered with straw mulch at a rate of 1 $kg.m^{-2}$ to minimise evaporation. The macro-fertilizer using DAP and KCl supplied by 22 kg N and 56 kg P_2O_5 ha^{-1} to all treatment plots and incorporated into the soil before sowing. Potassium was supplemented at 125 kg K_2O ha^{-1} and applied in five split applications at the 3–4 leaf stage, during flower bud formation, after the first harvest, 15 days after the third application, and following the subsequent flowering stage.

Soil samples were collected biweekly starting from the fifth week, at a depth of 0–20 cm. Five subsamples were taken per treatment plot using a diagonal sampling method and composited for analysis. Soil pH was measured in a 1:5 soil-water suspension using a calibrated pH probe (Mettler Toledo MP220) whilst electrical conductivity (EC) was determined in a 1:5 soil-water suspension using a conductivity probe (HI99300). Plant samples were collected at harvest to measure yield and dry biomass. Root and shoot samples were analysed for proline concentration, which served as an indicator of plant responses to saline and drought stresses. Proline content was determined to evaluate the salt tolerance of the crops. Immediately after harvest, root and shoot samples were collected to extract the amino acid proline using ninhydrin reagent and measured via UV-VIS absorption spectrophotometry (Carillo et al., 2011).

2.2 Effects of phosphate on the reproductive growth of cowpea during the 2023 and 2024 dry season

The effects of phosphorus on cowpea were examined in experiments conducted at the same field site during the 2023 (February to May) and 2024 dry season (February to June). The experiments followed a randomized completely block design (RCBD) with three replications. Two cowpea varieties, TN142 (coded as C1) and TLP69 (coded as C2), were used for the trials.

Phosphate fertilizer (single superphosphate) was applied at four rates of 0, 30, 60, and 90 kg P_2O_5 ha^{-1}. Nitrogen and potassium fertilizers (urea and KCl) were uniformly applied across all treatments at rates of 27.6 kg N and 45 kg K_2O ha^{-1}. Irrigation water was taken from nearby canals and farmers' reservoirs, with saline levels gradually increasing throughout the growing season due to saline intrusion. Irrigation timing and water volumes were determined according to traditional farmer practices, with the aim of maintaining relatively consistent soil moisture conditions across all plots.

The cowpeas were planted at a density of 51 plants per m^2. Raised beds approximately 30 cm high and 0.9 m wide were prepared for planting. Seeds were sown in two rows per bed with the distance of row x row = 70 cm and plant x plant = 30 cm. To minimize evaporation, all beds were covered with straw mulch at a rate of 1 kg.m^{-2}. Phosphate was applied at sowing across all treatment combinations while nitrogen and potassium were split into five applications at 10, 20, 30, 40, and 50 days after sowing (DAS).

2.3 Statistical analysis

All data were analysed using analysis of variance (ANOVA) appropriate for a randomized complete block design (RCBD). For the irrigation experiment (2022), a two-factor ANOVA was performed with irrigation regime and variety as fixed factors, and block included as a blocking factor in the model. For the phosphorus experiments (2023 and 2024), a two-factor ANOVA was conducted with phosphorus rate and variety as fixed factors, with block included as a blocking factor. When significant main or interaction effects were detected, treatment means were separated using Tukey's Honestly Significant Difference (HSD) test. Statistical analyses were performed using Minitab (Minitab, LLC, Pennsylvania, USA), and differences were considered statistically significant at $p \le 0.05$.

3. Results

3.1 Sensitivity of cowpea varieties to continuous and intermittent irrigations using saline water in field conditions

Table 1. *Effects of irrigation methods and cowpea varieties on biomass, yield, and proline content in shoots and roots of cowpea grown in the dry season of 2022.*

Treatment	Biomass (g.plant^{-1})	Yield (kg.ha^{-1})	Treatment	Proline	
				In shoot	In root
TN142 - I1	170.2 ± 34.2	1537.6 ± 230.4 bc	TN142	16.4 ± 4.1	12.3 ± 2.3
TN142 - I2	174.3 ± 23.0	742.3 ± 280.1 c			
TN142 - I3	188.3 ± 20.8	1151.0 ± 531.7 c			
TLP69 - I1	233.1 ± 74.4	2798.9 ± 349.5 a	TLP69	10.8 ± 1.1	5.9 ± 1.2

TLP69 - I2	223.1 ± 31.9	2349.3 ± 459.5 ab			
TLP 69 - I3	200.8 ± 40.8	1560.3 ± 1320.9 abc			
P value	NS	0.00	P value	0.037	0.002

* Values in the same column followed by the same letters are not significantly different at the 5% level.

The three irrigation methods (continuous, intermittent, and the farmer's traditional practice) did not signfincantly affect plant biomass (Table 2) for either cowpea variety, although differences in yield were observed. For the TN142 variety, yield was higher under the farmer’s practice irrigation treatment compared with the continuous and intermittent irrigation treatments; however, these differences were not statistically significant. Similarly, TLP69 achieved its highest yield under the farmer’s practice irrigation, but irrigation method did not significantly influence yield within this variety. The only significant irrigation effect was observed between the farmer’s practice and continuous irrigation treatments when comparing TLP69 and TN142. Analysis of proline concentration indicated that TN142 accumulated significantly higher proline levels in both shoot and root tissues than TLP69 (Table 2).

Table 2. *Soil EC measurements from cowpea crop in the dry season of 2022*

Treatment	Soil EC at			
	Week 5	Week 6	Week 7	Week 8
TN142 - I1	501.8 ± 38.7 a	434.3 ± 21.4 a	359.5 ± 34.1 b	358.3 ± 34.8 b
TN142 - I2	501.8 ± 30.9 a	412.8 ± 22.0 b	360.8 ± 16.1 bc	334.5 ± 40.3 c
TN142 - I3	463.3 ± 40.7 a	424.0 ± 48.2 ab	371.5 ± 39.5 bc	324.8 ± 23.3 c
TLP69 - I1	449.5 ± 52.2 a	423.5 ± 42.9 ab	365.0 ± 32.9 bc	322.3 ± 17.0 c
TLP69 - I2	491.3 ± 71.9 a	427.8 ± 17.2 ab	354.3 ± 47.7 b	346.3 ± 53.8 b
TLP69 - I3	488.3 ± 76.4 a	426.3 ± 19.8 ab	387.5 ± 31.7 b	353.8 ± 42.7 b
P value	0.000	0.000	0.002	0.010

* Values in the same column followed by the same letters are not significantly different at the 5% level.

At each sampling week (weeks 5–8), significant differences in soil electrical conductivity (EC) were detected among treatment combinations ($p < 0.05$) (Table 2). However, across all irrigation treatments and varieties, soil EC consistently declined over time. At week 5, EC values ranged from 449 to 501, whereas by week 8 they had decreased to between 322 and 358. This pattern indicates a general temporal reduction in soil salinity during the latter stages of the growing season, irrespective of irrigation regime.

3.2 Effects of phosphorus application on the growth of cowpea TN142 and TLP69 during the 2023 dry season

Cowpea grown in the 2023 dry season showed a clear response to P application for both plant biomass and yield (Figure 1). For the TN142 variety, lower P fertilizer rates (30 and 60 kg P_2O_5 ha^{-1}) resulted in reduced yields compared with the control, whereas the highest application rate (90 kg

P_2O_5 ha^{-1}) produced a significant yield increase. The TLP69 variety achieved the highest yield among all treatments with a maximum of 1,867 kg/ha recorded at 90 kg P_2O_5 ha^{-1}.

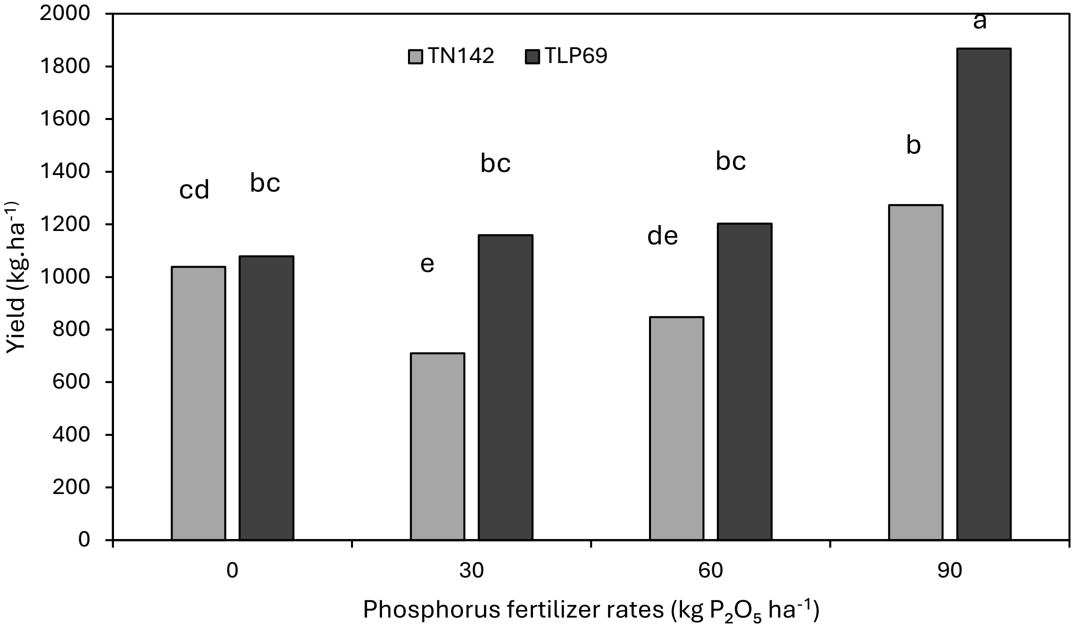

Figure 1. *The effect of increasing phosphorus fertiliser rates (0, 30, 60 and 90 kg P_2O_5 ha^{-1}) on the yield of two different cowpea varieties (TN 142 and TLP 69) during the 2023 dry season. Differing letters indicate significant difference at p=0.05.*

Dry plant biomass responded positively to P application, with increasing fertiliser rates resulting in greater biomass accumulation. The highest P rate (90 kg P_2O_5 ha^{-1}) produced the greatest dry biomass measurements for TN142 (26.3 g.plant^{-1}) and TLP69 (25.6 g.plant^{-1}), with no significant difference observed between the two varieties. It must be noted that an incomplete harvest occurred due to unseasonal flooding interrupting the indeterminate fruiting pattern of cowpea and causing early termination of the trial. Thus, it is premature to draw definitive conclusions about the effects of fertilizer concentration on this variety and repetition of the experiment in subsequent growing seasons is necessary to more reliably assess the influence of phosphorus application on cowpea yield and reproductive development.

Table 3. *Effects of different phosphorus fertilizer rates on the growth of two different cowpea varieties for the 2023 dry season.*

Treatment	**Fresh stem and leaf weight (g.plant^{-1})**	**Dry stem and leaf weight (g.plant^{-1})**
TN142		
P0	103.4 ± 5.0 ab	19.2 ± 1.9 d
P1	99.1 ± 9.5 ab	20.5 ± 3.2 bcd
P2	113.2 ± 4.3 ab	25.8± 1.6 a

P3	118.5 ± 7.8 a	26.3 ± 2.5 a
TLP69		
P0	95.9 ± 8.6 b	17.9 ± 1.2 d
P1	112.5 ± 5.7 ab	20.0 ± 1.7 cd
P2	115.6 ± 8.3 ab	24.9 ± 0.8 abc
P3	112.4 ± 8.7 ab	25.6 ± 1.0 ab

* Values in the same column followed by the same letters are not significantly different at the 5% level.

3.2. Effects of phosphorus application on the growth of cowpea TN142 and TLP69 during the 2024 dry season

Phosphorus application also had a positive effect on cowpea growth in the 2024 dry season (Figure 2). Cowpea yield was highly responsive to phosphorus application, with the highest yield recorded for TLP69 at 5.4 t/ha under the 90 kg P_2O_5 ha^{-1} treatment. Both varieties achieved their greatest yields at the higher P application rates (60 and 90 kg P_2O_5 ha^{-1}). Notably, the control treatment of TLP69, which received no P fertilizer, produced a higher yield than all TN142 treatments.

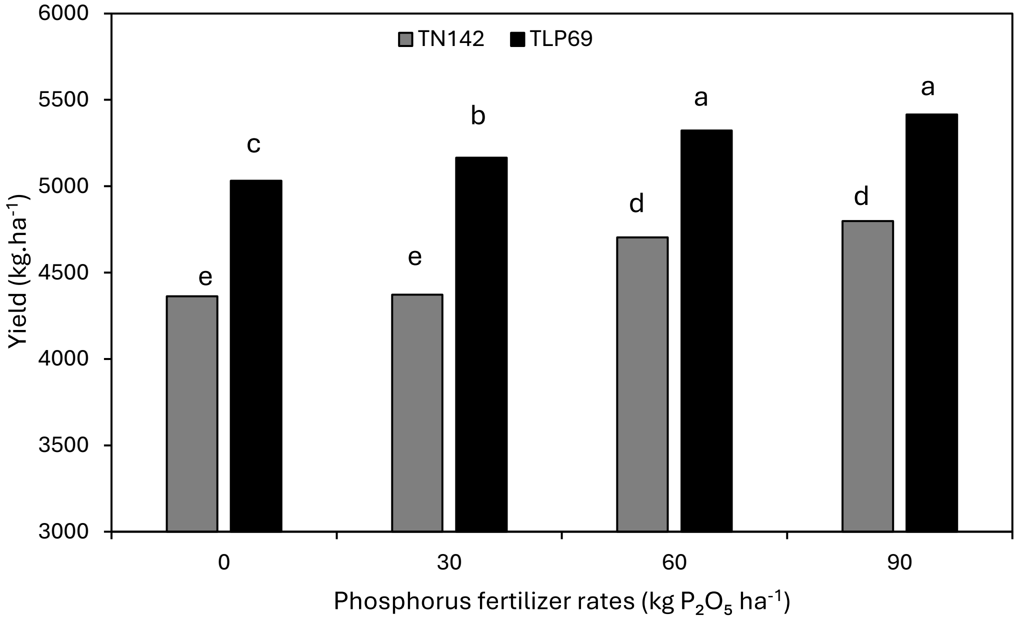

Figure 2. *The effect of increasing phosphorus fertiliser rates (0, 30, 60 and 90 kg P_2O_5 ha^{-1}) on the yield of two different cowpea varieties (TN 142 and TLP 69) during the 2024 dry season. Differing letters indicate significant difference at p=0.05.*

Plant biomass (dried stem and leaf weights) increased with higher fertiliser rates (60 to 90 kg P_2O_5 ha^{-1}) for both varieties, although no effect of fertiliser rate was reported on root weights (Table 4). It is important to note that the 2024 dry season yields were harvested at the end of the fruiting phase, whereas the 2023 season faced weather-related constraints that required early harvests and premature termination of experiments.

Table 4. Effects of different phosphorus fertiliser rates on cowpea growth parameters for the 2023 dry season

Treatment	**Plant height**	**The weight of**			
		Fresh stem and leaf	**Dry stem and leaf**	**Fresh root**	**Dry root**
	(cm)	**(g.plant^{-1})**			
TN142					
P0	163.5 ± 17.5	82.6 ± 5.4 b	15.8 ± 1 b	5.9 ± 0.6	1.7 ± 0.2
P1	165.9 ± 37.5	80.4 ± 5.1 b	14.9 ± 0.9 b	6.1 ± 0.3	1.8 ± 0.1
P2	167.3 ± 19.6	111.5 ± 6.6 a	21.8 ± 1.1 a	6.6 ± 0.3	2.0 ± 0.3
P3	180.4 ± 41.9	122.0 ± 3.8 a	23.5 ± 1.0 a	6.6 ± 0.3	2.1 ± 0.3
TLP69					
P0	162.7 ± 30.8	87.5 ± 7.6 b	18.1 ± 1.3 b	6.3 ± 0.5	2.0 ± 0.2
P1	179.0 ± 30.9	92.0 ± 6.7 b	17.4 ± 1.4 b	6.0 ± 0.5	1.8 ± 0.1
P2	186.7 ± 45.8	118.4 ± 7 a	22.0 ± 1.3 a	6.4 ± 0.3	1.9 ± 0.2
P3	181.3 ± 28.1	121.7 ± 5.2 a	22.2 ± 1 a	6.8 ± 0.2	2.1 ± 0.2

* Values in the same column followed by the same letters are not significantly different at the 5% level.

4. Discussion

4.1 Using irrigation deficit on cowpea

Although continuous, intermittent, and farmer-practice irrigation methods did not significantly affect biomass accumulation in either variety, yield trends favoured the farmer's traditional irrigation practice, particularly for TN142 (Table 2). Flood-based irrigation techniques familiar to farmers producing rice can result in inefficient water application to upland crops and increase the risk of waterlogging (Borrell and Cooten, 2001). In this study, the farmer-practice irrigation regime may have coincidentally supplied water during critical reproductive stages, thereby supporting yield formation. However, this approach is typically characterised by relatively high water inputs and limited scheduling precision. Continued reliance on abundant irrigation under such practices raises concerns about inefficient water use, accelerated depletion of freshwater resources, and the potential risk of transient waterlogging stress, particularly in the context of increasing drought frequency and water scarcity in the Mekong River Delta (Nghia et al., 2024). Despite the trend towards higher yields under the farmer-practice irrigation, the trial demonstrated that intermittent irrigation did not result in significant yield penalties. This suggests that intermittent irrigation could be a viable management strategy for maintaining cowpea productivity while improving water-use efficiency and reducing overall water consumption in water-limited regions of the Mekong River Delta. The lack of strong yield responses to continuous or intermittent irrigation may also reflect cowpea's inherent drought tolerance (Sansa et al., 2025) and ability to maintain biomass under varying moisture conditions.

Cowpea's growth is relatively resilient to variations in water application, but its reproductive performance is more sensitive to irrigation regime, particularly under stress-prone conditions (Lima et al., 2019, Oumarou et al., 2015). This aligns with field studies showing that cowpea yield can be more strongly influenced by water availability during critical reproductive stages than by vegetative growth, and that moderate water deficits often have limited impacts on biomass but reduce reproductive success if they coincide with flowering and pod filling (Poudel et al., 2025).

Proline accumulation is a well-documented physiological response to water and salinity stress in cowpea and other legumes, where it functions as an osmo-protectant and stress indicator under drought and saline conditions (Al-Maskari and Yaish, 2025). In this study, TN142 consistently accumulated significantly higher proline levels in both shoots and roots compared with TLP69, despite similar biomass outcomes, suggesting that TN142 experienced greater osmotic stress under the imposed irrigation treatments. This pattern aligns with previous research showing that increased proline concentrations are associated with osmotic adjustment, maintenance of cell turgor, and protection of cellular systems under water and salinity stress (Zegaoui et al., 2017, Carvalho et al., 2019). In contrast, the relatively lower proline levels in TLP69, coupled with its stable yield performance across irrigation regimes, may reflect greater stress tolerance or more efficient water-use strategies in this variety.

Taken together, these results imply that intermittent irrigation strategies, combined with varietal selection for lower stress-induced proline accumulation, may help optimise cowpea yield in areas of the MRD characterised by salinity and intermittent water supply.

The soil EC values declined over time, despite progressively saline irrigation conditions during the dry season (Table 2). This pattern contrasts with the expectation that soil salinity would gradually accumulate under irrigation with saline water. A plausible explanation for this unexpected decline is related to seasonal rainfall dynamics (Figure 3).

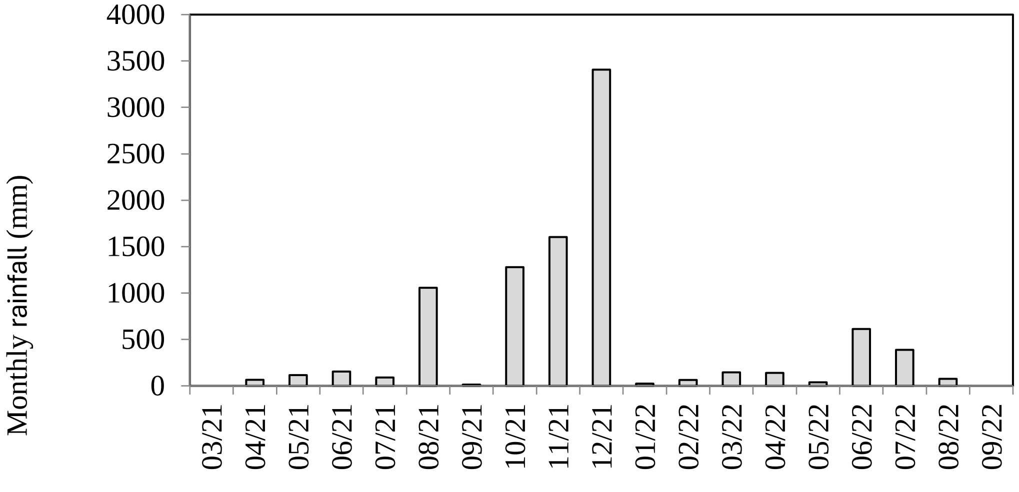

Figure 3. *Total monthly rainfall (mm) for the 2021-2022 field trial at Lieu Tu commune, Tran De district, Soc Trang province, Vietnam.*

Figure 3 presents total monthly rainfall during the 2021–2022 field trial at Lieu Tu commune, Tran De district, Soc Trang province, Vietnam. The data indicate a prolonged dry period from January to May 2022, with minimal precipitation. However, a marked transition occurred in June 2022, when rainfall exceeded 500 mm. The substantial rainfall at the end of the experimental period likely promoted leaching and dilution of soluble salts from the upper soil profile. This flushing effect would have reduced surface soil EC, thereby explaining the observed decline in measured salinity toward the final sampling weeks.

4.2 Cowpea response to phosphorus fertiliser management

Findings from this study reinforce the well-established role of phosphorus as a critical nutrient for cowpea growth, biomass accumulation, and yield development (Suzuki et al., 2022) in the novel environment of a saline and drought affected MRD. In both the 2023 and 2024 dry-season trials, higher P application rates (especially 60–90 kg P_2O_5 ha^{-1}) were associated with increased dry biomass and grain yield, indicating that P availability can strongly limit productive performance in cowpea under dry, saline, and nutrient-poor conditions. These results are consistent with previous field studies showing that P fertiliser enhances vegetative growth, nodulation and yield components in cowpea (Kyei-Boahen et al., 2017, Bawa, 2020), with optimal application rates at 60kg P ha^{-1} (Karikari et al., 2015). Phosphorus influences root development, energy transfer, and symbiotic nitrogen fixation — processes that underpin successful reproductive growth in legumes — and its deficiency has been widely identified as a constraint in cowpea production systems across diverse agro-ecological zones (Karikari et al., 2015, Mathew et al., 2024, Augustine and Godfre, 2019). The comparatively high yield of TLP69 even in the absence of applied P suggests potential varietal differences in P-use efficiency, a trait that could be exploited in breeding and management strategies for low-input systems (Augustine and Godfre, 2019). However, the influence of seasonal factors, such as complete fruiting in 2024 versus the interrupted harvest of 2023, highlights the importance of environmental conditions in expressing the benefits of P fertilisation and underscores the value of multi-season trials to robustly evaluate nutrient responses.

5. Conclusion

This study demonstrates that cowpea can be successfully produced under the saline and water-limited conditions of the MRD, provided that irrigation and phosphorus fertiliser management are appropriately optimised. While biomass accumulation was largely unaffected by irrigation regime, yield responses were more sensitive to water management, particularly during reproductive stages. Although farmer-practice irrigation tended to support higher yields, intermittent irrigation achieved comparable productivity without significant yield penalties, highlighting its potential as a more water-efficient alternative in regions facing increasing water scarcity. Physiological responses further revealed important varietal differences in stress tolerance with lower proline levels for TLP69 alongside more stable yield performance, suggesting superior stress resilience and water-use efficiency.

Phosphorus fertilisation emerged as a key driver of cowpea productivity where higher P application rates consistently enhanced biomass and grain yield across seasons, confirming that P availability is a major constraint under dry, saline, and nutrient-poor conditions. The strong performance of TLP69 under low-P conditions further suggests the presence of varietal differences in phosphorus-use efficiency, offering opportunities for low-input production systems. Overall, the results indicate that combining intermittent irrigation strategies with adequate phosphorus fertilisation and stress-tolerant, nutrient-efficient cowpea varieties can improve water-use efficiency and sustain yields in the MRD. These findings provide a foundation for the development of climate-resilient cowpea management practices and support the crop's potential as an alternative option for farmers adapting to increasing salinity and water scarcity.

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Paper 3: Application of rice straw as a soil surface mulch decreases soil salinity and increases the production of beetroot and maize on low elevation saline soils in the Mekong Delta of Vietnam

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Abstract

Salinity is becoming a major threat to the growth of continuous rice crops in the low-elevation Vietnamese Mekong Delta. Seawater is increasingly permeating up the river and irrigation water distribution systems in the dry season. There is insufficient knowledge of possible alternatives to rice and management practices during this season to maintain crop production. We assessed the growth of beetroot and maize and tested the value of rice-straw mulch at four rates (0, 3.5, 7.0 and 10.5 t ha^{-1}) at three sites over two years. At the two higher elevation locations (Lieu Tu and Long Phu, 0.80 and 0.96 m above mean sea level (amsl), respectively) dry season crops were successfully grown for two years and high levels of production were possible with beetroot and maize (average commercial crop yields up to 42 and 5.4 t ha^{-1}, respectively) while crops could not be established at the low elevation site (Long My, 0.21 m amsl) due to inundation effects. For beetroot and maize, application of mulch increased commercial crop yields by up to 114% and 49%, respectively, decreased the concentrations of Na^+ in the shoots of plants at harvest by 19% and 37%, respectively, and decreased the salinity ($EC_{1:5}$) of the topsoil (0-15 cm) by 13%. Mulch effects were maximized at an application rate of 7.0 t ha^{-1}. Analysis of relationships between soil salinities at different depths and yield, and between mulch effects and yield suggested that the rooting depth of beetroot may have been constrained at these sites compared with maize. Very shallow water tables (~20 cm depth) were implicated as the likely cause of this constraint. In overview, our work suggests that exceptional yields of beetroot and maize are possible as alternative crops to rice in this delta environment where salinity and waterlogging are key risks.

Keywords: climate change, sea-level rise, sodium concentrations in leaves, soil moisture, solute potential, water deficit.

1. Introduction

Salinization of soil and freshwater scarcity (drought) are becoming significant issues constraining production in the Mekong Delta of Vietnam (Kaveney et al. 2023). The application of surface soil mulches and the growth of salt-tolerant crops have the potential to substantially improve outcomes for farmers in this landscape (Fig. 1).

The application of soil surface mulches has been used to improve crop growth on land at risk of salinity in a variety of landscapes (Aragüés et al. 2014; Paul et al. 2020). Mulches can suppress weeds (Johnson et al. 2004; Jodaugienė et al. 2006; Ramakrishna et al. 2006) and alter soil temperatures (Dong et al. 2009; Adhikari et al. 2016). In addition, they can decrease the evaporation of water from the soil surface, thereby maintaining soil hydration, and can also reduce the movement of salt to the soil surface by capillarity (Paul et al. 2020).

The combination of these two effects increases (i.e. makes less negative) soil solute potential, which enables better crop growth in saline soils (Paul et al. 2020). As it is not the soil salinity (of which the EC_e or $EC_{1:5}$ is measured) that affects growth, but rather the salinity of the soil solution (Setter et al. 2016), estimations of solute potential (Ψ_s) may be a better parameter than measures of soil salinity in accounting for variation in plant growth. Solute potential is an analogue of the salinity of the soil solution and has the advantage that it can be added to soil matric potential (Ψ_m) to provide an estimate of the total water potential (Ψ) affecting the plant. Ψ_s is calculated from the $EC_{1:5}$ (dS m^{-1}) and the soil gravimetric water content (W; % dry mass) (Paul et al. 2020) as:

$$\Psi_s = -22.58 * EC_{1:5} / \text{gravimetric soil water content (\%)}$$

Rice straw has considerable potential as a soil surface mulch in areas at risk of salinity in Asian deltas, as rice is a staple crop in these areas and straw is its major by-product (Paul et al. 2020; Sarangi et al. 2010, 2021). Rice is also a major staple crop in Vietnam, but the role of rice straw as a mulch has not yet been examined here. Currently, much of the rice straw produced in the Mekong Delta is either burned after harvesting or used for mushroom cultivation (Hong Van et al. 2014).

Salinity is becoming a major threat to agriculture in the Mekong Delta because river flows are seasonal, high during the wet season but much lower during the dry season. During times of low river flow, seawater intrudes up the rivers and waterways of the delta, causing land and water salinity (Kaveney et al. 2023; Minderhoud et al. 2019). Rice is the dominant crop in the delta and, where water is available, three crops are generally grown in three seasons, the wet He Thu and Thu Dong seasons, and in the dry Dong Xuan season. Unfortunately, rice is sensitive to salinity and often fails in the dry season: the search has therefore begun to identify suitable alternative salt-tolerant crops and better soil management strategies to enable cropping to continue in the dry season (Kaveney et al. 2023).

For most glycophytic (salt-sensitive) crops, experiments conducted under controlled conditions have shown negative relationships between soil salinity and yield, positive relationships between soil salinity and concentrations of Na^+ in the leaves, and negative relationships between concentrations of Na^+ in the leaves and yield (see reviews by Greenway and Munns 1980; Munns and Tester 2008). Increased Na^+ uptake by leaf cells in glycophytes is a problem because it impairs K^+ metabolism (Shabala and Cuin 2008). These relationships are harder to see in field experiments because salinity is more difficult to control in the field.

In the dry Dong Xuan season, water availability is limited by a lack of rainfall and loss of low-salinity (4 g L^{-1}) irrigation water (Kaveney et al. 2023). These drought conditions typically worsen as the season progresses through to April and May, greatly harming plants and decreasing crop yield (Phung et al. 2020). Additionally, early in the dry season, the soils of the Mekong Delta can also be subject to another critical stress. Their low elevation (mostly < 1.5 m above sea level; Minderhoud et al. 2019) means that they are poorly drained and, if unseasonal rain falls, they can become waterlogged. Waterlogging is a particularly damaging stress because it causes soils to become oxygen deficient, generally within 1-2 days (Belford et al. 1985; Barrett-Lennard et al. 1986), which adversely affects root metabolism, growth and survival (Gibbs and Greenway 2003). In saline landscapes, waterlogging can also increase the uptake of Na^+ and Cl^- and decrease the uptake of K^+, which exacerbates salinity damage to plants (Barrett-Lennard 2003; Barrett-Lennard and Shabala, 2013).

Beetroot (*Beta vulgaris* subsp. *vulgaris* Conditiva Group) and maize (*Zea mays* L.) are relevant test species as alternative crops to rice. Beetroot is ranked as being moderately tolerant to salinity (Maas and Grattan 1999) and is descended from a salt-tolerant ancestor, marine beet (*Beta maritima*; Yolcu et al. 2021). By contrast, maize is ranked as being moderately sensitive to salinity (Maas and Grattan 1999). However, the situation with the relative waterlogging tolerance of these crops is quite different. Members of the grass family (Poaceae) have tolerance to waterlogging because they form crown (nodal) roots that can produce aerenchyma (continuous pores) that allow for the internal diffusion of oxygen from shoots to roots (Armstrong 1979); maize is able to form aerenchyma in its crown roots (Drew et al. 1980). By contrast, beetroot is not known to form any type of aerenchyma (Rojas-Méndez et al. 2021). It can therefore be expected to be waterlogging sensitive.

The feasibility and performance of beetroot and maize as dry-season alternatives to rice and the effects of rice-straw mulch on their growth have been insufficiently studied. Therefore, the research conducted in this paper aimed to determine if the growth of alternative crops to rice was possible in saline environments in the Mekong Delta and whether their growth could be increased through the use of straw mulch. We had four hypotheses. Firstly, the use of rice straw mulches would increase the commercial yield of alternative crops (beetroot and maize) (H1). Secondly, these mulches would decrease soil salinity and increase soil water content, thereby increasing (making less negative) soil solute potentials (H2). Thirdly, these mulches would improve the ion relations of the plants, decreasing Na^+ in the shoots (H3). Fourthly, there would be relationships between soil conditions, ion concentrations in leaves, and yield (H4).

2 Materials and Methods

2.1 Location of trials

The field trials in this study were conducted in the dry season (2021 and 2022) at three locations in Long Phu (9°34’56”N, 106°7’48”E) and Lieu Tu (9°28’27”N, 106°6’6”E; Soc Trang Province) and Long My (9°40'59"N 105°23'46"E; Hau Giang Province) of the Vietnamese Mekong Delta (Fig. 2). Based on the FAO/UNESCO World Soil Resources Classification System (IUSS Working Group WRB 2007), the soils at the Lieu Tu and Long Phu experimental sites are Eutric GLEYSOLS (euGL) and Mollic GLEYSOLS (moGL), respectively. All sites had low elevation (< 1 m height above mean sea level). Although relatively close to the coast, the sites at Long Phu and Lieu Tu were more elevated (0.96 and 0.80 m above mean sea level, respectively (amsl)) than the less elevated (0.21 m amsl) inland site at Long My (Minderhoud et al. 2019).

The area where the research was conducted has a tropical monsoon climate, with a distinct dry season from November to March and a rainy season from April to October. Annual rainfall was ~1900 and 2250 mm in 2021 and 2022, respectively (Fig. S1A). Temperatures were relatively similar year-round. Minimum and maximum average monthly temperatures fell in the range 19-24 and 31-36°C, respectively (Fig. S1B). Weather conditions of Long My site had not been reported here because the trial failed (see Supplementary Materials Fig. S1).

2.2 Experimental design

The experiments at Lieu Tu and Long Phu had four possible crop options; these were fallow, beetroot (*Beta vulgaris* subsp. *Vulgaris* 'Conditiva Group'), maize (*Zea mays* L. ssp. *mays*), and either soybean (Lieu Tu) or watermelon (Long Phu); each crop had 3 replicates. The plantings were repeated on the same plots within a rice/rice/alternative crop rotation for two growing seasons. Within each plot there were 4 rice straw mulch treatments (subplots); these were 0, 3.5, 7.0 and 10.5 t ha^{-1}. The rates represent practical rates possible based on rice straw production (as influenced by rice yield and harvest index) and have been used in Southern Bangladesh (Paul et al. 2020). This paper focuses on the yields of the crops grown at both sites (beetroot and maize); the yield results for soybean (Lieu Tu) and watermelon (Long Phu) are reported in the Supplementary Material (Table S1).

2.3 Experimental layout and irrigation procedure

The major dates associated with crop establishment, harvesting and other field activities are reported in the Supplementary Materials (Table S2).

Each plot had an area of 25 m^2 with dimensions of 5 x 5 m. The plants were established onto 15 cm high raised beds that were 1.2 m wide, with irrigation channels on either side. Ditches (20 cm wide x 20 cm deep) were made to minimize waterlogging at the soil surface. Between the blocks, there were irrigation and drainage channels (40 cm wide x 50 cm deep). For maize, plants were in two rows per bed at a planting density of 5 plants m^{-2}. For beetroot, the plants were in 4 rows per bed at a planting density of 16 seedlings m^{-2}.

Maize and beetroot seeds were soaked in water for 24 h. The seeds were then incubated in the dark for 72 h. The germinated seeds were then planted into the field.

The plants were irrigated with local river water once a day at about 4 pm. The exception to this was at Lieu Tu in 2021 when irrigation was stopped between 1 March (45 days after sowing) and 16 Mar (60 days after sowing) because the salinity of canal water had exceeded the threshold for acceptable salinity, i.e. 4 g L^{-1}. Irrigation water gradually became more saline during each growing season, particularly at Lieu Tu. At the outset, the salinity of the irrigation water (EC_w) was 1-2 dS m^{-1}, but by early March the EC_w had increased to 2-5 dS m^{-1} (see Supplementary Materials Fig. S2).

Organic fertilizer (compost 5 t ha^{-1}) and lime (2 t ha^{-1}) were applied as a basal application at the stage of land preparation. The soils were fertilized with 180-70-70 kg NPK ha^{-1} for maize; and 105-60-150 kg NPK ha^{-1} for beetroot, in even splits order (0, 10, 30 and 40 days after crop established). The four rice straw mulch rates were applied to the surface of the subplot beds as experimental treatments immediately after sowing.

2.4 Harvest procedure

Maize and beetroot were harvested 68-75 and 81-92 days after sowing (see Supplementary Materials, Table S2). Plant samples were collected at harvest to analyze the Na and K contents in mixed stem and leaf samples. The analyses of soil and plant materials also contained standard soils and plant samples with known attributes to confirm the consistency of the analytical techniques. Plants were harvested from a 1.2 m length of raised bed at three locations in each subplot. This gave a total harvested area of 4.32 m^2 per subplot.

Maize plants were cut at ground-level and then separated into ears of maize with their enclosing leaves (the commercial product), and other shoot materials (leaves and stems). These were fresh weighed in the field. Composite leaf and stem material from 3 plants and the ears from three plants (one from each subplot location) were taken back to the lab where these were oven dried at 70°C to constant weight and mechanically ground to a fine powder (< 2 mm diameter). Samples were digested by placing 0.3 g of powdered plant sample into an Erlenmeyer flask and mixing with 3 mL of the digestion solution (18 mL of deionized water, 100 mL of sulfuric acid and 6 g of salicylic acid) at 180°C. After further digestion with H_2O_2, the sample solutions were diluted, and Na and K concentrations in the solutions were measured by atomic absorption spectrometry using an AA-7000 instrument (Shimadzu Corporation) (Sparks et al. 1996).

Beetroot plants were uprooted and separated into the roots and the above ground biomass (leaves and stems). The roots (the commercial product) were washed in the field, lightly air-dried and fresh weighed. The shoot subsampling procedure was that the above ground biomass of 2 plants was taken from each of three locations in the subplot (6 plants total). These were taken back to the lab and oven dried at 70°C, mechanically ground and extracted as described above.

2.5 Collection and analysis of soil and water samples

A preliminary survey of surface soil was conducted at each site in January 2021 prior to sowing (see Supplementary Material, Table S3). After crop establishment, major soil samplings were conducted mid-way through the growing season and at the time of harvest. For these samplings, six auger holes were made in each subplot and the soil from these was amalgamated into 3 composite samples at 0–15, 15–30 and 30–40 cm depth. The soils were taken to the lab, weighed, oven-dried at 40°C until they reached a constant weight, and were then weighed again. The weights before and after oven drying at 105°C were used to calculate gravimetric soil moisture content. Soils were then ground using a mortar and pestle, and subsamples were taken for the measurement of salinity ($EC_{1:5}$; HM COM-100). The $EC_{1:5}$ and soil water content values were used to estimate soil solute potential (Ψ_s).

Soluble Na^+, and K^+ were determined by extracting soil with deionized water at a ratio of 1:5 (soil:water) and shaking for 1 hour at 120 round per minute (rpm) and determined with AA-7000. Extractable cations were analyzed by extracting a soil sample (2.5 g) three times with 0.1 M $BaCl_2$ solution, each time with 30 mL, and shaking for 1 hour at 120 rpm, then made up to a total volume of 100 mL and determined with AA-7000. Exchangeable Na^+ and K^+ were obtained by subtracting soluble cations from extractable cations. Some additional soil measurements are reported in the Supplementary Material Table S3. Methods of analysis have been presented in the footnote of the table. The analyses of soils also contained standard soil samples with known attributes to confirm the consistency of the analytical techniques.

The salinity of the irrigation water (EC_w) was measured at weekly intervals to decide on the suitability of the daily watering regimes.

2.6 Statistical analysis

Statistical analyses were conducted using GENSTAT 22nd edition (VSNi).

The normality of datasets was tested using the Shapiro-Wilk test. All datasets were normally distributed except the yield of maize. Prior to conducting ANOVAs, maize data were transformed using a natural logarithmic transformation; treatment means were back transformed for presentation.

To determine the effects of level of mulch and site on the commercial yield of beetroot and maize (Table 3) a repeating measures analysis of variance (ANOVA) was conducted. In this, year of trial (2021 or 2022) was treated as the within-subjects (repeating) measure.

To determine the effects of level of mulch, site and time on the $EC_{1:5}$, soil water content and soil solute potential (Table 4), a repeating measures ANOVA was conducted. In this, soil depth was treated as the within-subjects (repeating) measure. These analyses included the data from all subplots.

To determine the effects of level of mulch, site and time on the concentrations of Na^+ and K^+ in shoots of beetroot and maize (Figure 6) a repeating measures ANOVA was conducted. In this, year of trial (2021 or 2022) was treated as the within-subjects (repeating) measure.

Treatment means were compared with reference to the least significant difference ($LSD_{0.05}$). Means were considered different if the difference between means was greater than the $LSD_{0.05}$.

Correlations between soil conditions ($EC_{1:5}$, soil water content and solute potential at 3 depths in the soil profile), yield and Na^+ in the leaves were conducted in Jamovi version 1.6.23.0 and drawn in EXCEL.

3 Results

These results are in five sections. The first provides an overview of our trials with alternative crops conducted in 2021 and 2022 at the three locations, Long Phu and Lieu Tu (in Soc Trang Province) and Long My (in Hau Giang Province). The second examines the effects of variables (including mulch treatment) on the commercial yield of the beetroot and maize (H1) at the two sites. The third examines the effects of variables (including mulch treatment) on soil conditions (H2). The fourth examines the effects of variables on shoot ion contents (H3). Finally, the fifth section examines the relationships between soil conditions, ion concentrations in the shoots and commercial yield (H4).

3.1 Overview of trials on adaptation of alternative crops

The growth of maize and beetroot was successful at Long Phu and Lieu Tu, but the crops could not be planted in a timely manner at Long My; this was because the latter site was inundated by excessive rainfall and lacked internal drainage (Table 1). Watermelon was trialed at Long Phu and was partly successful. Soybean was trialed at Lieu Tu and had low productivity. The yield data for these crops are given in the Supplementary Materials (Table S1).

3.2 Effects of variables on the yield of beetroot and maize (H1)

The rate of mulch applied significantly influenced the commercial yield of beetroot and maize and an interaction between site and year also occurred for the yield of each crop (Fig. 3).

3.2.1 Mulch effects

Mulch had strong benefits on the yield of beetroot and maize. With no mulch, average yields of beetroot and maize were 19.4 and 3.6 t ha^{-1} respectively. Relative to the un-mulched control, beetroot yields increased by 18, 97 and 114% with the addition of 3.5, 7.0 and 10.5 t ha^{-1} of mulch, respectively. With maize, the equivalent yield gains were 16, 44 and 49% with 3.5, 7.0 and 10.5 t ha^{-1} of mulch, respectively. For beetroot, there were continuing benefits to yield of increasing mulch application, with the highest average yield being 41.5 t ha^{-1}; however, maize, had statistically similar average yields (5.2–5.4 t ha^{-1}) with mulch applications of 7.0 and 10.5 t ha^{-1} (Fig. 3 A, C).

3.2.2 Site x year effects

For beetroot, highest average yields occurred at Long Phu in 2021 (45.6 t ha^{-1}), and lowest average yields occurred at Lieu Tu in 2021 (18.6 t ha^{-1}). For maize, highest yields occurred at Long Phu in 2022 (5.1 t ha^{-1}), and lowest yields occurred at Lieu Tu in 2021 (3.8 t ha^{-1}) (Fig. 3 B, D). The site x year effects were at least partly caused by changes to the irrigation schedule. At Lieu Tu in 2021 irrigation ceased for a period of 2 weeks due to a lack of fresh water in the local canal. This had stronger adverse effects on shallow-rooted beetroot than on deeper-rooted maize (Fig 3 B, D). Despite this, the factors affecting the yield of beetroot and maize were systematic. Across all replicates of mulch treatment, site and year, the yields of maize and beetroot were significantly correlated with each other (Fig. 4).

3.3 Effects of variables on soil (H2)

Soil $EC_{1:5}$ was significantly ($P < 0.001$) affected by mulch, soil depth, site and time (Fig. 5). Soil solute potentials were similarly affected by these variables. Mulch had no significant effect on soil water content, but this was affected by site, time, and depth. These main effects of the variables and selected interactions are reported in the Supplementary Materials, Table S5.

3.3.1 Depth effects

$EC_{1:5}$ was significantly ($P < 0.001$) affected by soil depth. Across all plots, $EC_{1:5}$ values ranged (5th to 95th percentile) between 0.21 and 0.83 dS m^{-1} at 0–15 cm, and between 0.22 and 0.50 dS m^{-1} at the other depths sampled (15–30 and 30–40 cm). On average, $EC_{1:5}$ values were 55% higher at 0–15 cm than at 15–30 cm, but values at 15–30 cm and 30–40 cm were similar (Fig. 5A and C; Supplementary Materials Table S5).

3.3.2 Mulch effects

Mulch significantly decreased average $EC_{1:5}$ ($P < 0.001$) and there was an interaction between mulch and depth ($P < 0.05$). The ameliorative effects of increasing mulch on $EC_{1:5}$ occurred at 0-15 cm but did not occur at the deeper depths sampled (15-30 and 30-40 cm). At 0-15 cm, application of 7.0 t ha^{-1} mulch decreased $EC_{1:5}$ by 13% compared with unmulched controls, and there was no further benefit of additional mulch at 10.5 t ha^{-1} (Fig. 5A).

3.3.3 Time effects

Time had significant ($P < 0.001$) effects on $EC_{1:5}$. On average, $EC_{1:5}$ values were 40% higher in 2021 than in 2022, but $EC_{1:5}$ values also increased by 3-10% between the mid-season and the end of the growing season (Fig. 5B).

3.3.4 Site effects

Site had a significant ($P < 0.001$) main effect on $EC_{1:5}$. On average, $EC_{1:5}$ values were 24% lower at Long Phu than at Lieu Tu. These site differences occurred at all depths measured (Fig. 5C).

3.4 Effects of variables on shoot ion contents (H3)

The Na^+ concentrations in the shoots differed substantially between the two crops: the range of concentrations (5th to 95th percentile) in beetroot was 132–351 cmol kg^{-1}, about an order of magnitude higher than in maize (15–39 cmol kg^{-1}).

3.4.1 Mulch effects

For each crop, there were significant effects of mulch on Na^+ in the shoots ($P < 0.001$ for beetroot; $P < 0.01$ for maize). In beetroot, the average Na^+ concentration was about 240 cmol kg^{-1} without mulch, and the application of mulch at 10.5 t ha^{-1} decreased this concentration by 19% (Fig. 6A). In maize, the average Na^+ concentration was about 30 cmol kg^{-1} without mulch, and the application of mulch at 10.5 t ha^{-1} decreased this concentration by 37% (Fig. 6C). Mulch had a significant effect on K^+ in shoots in beetroot ($P < 0.05$) but not in maize (Fig. 6B, D). In beetroot the highest rate of mulch (10.5 t ha^{-1}) increased K^+ concentrations by ~13% (Fig. 6 B).

3.4.2 Site effects

Site had a significant effect on Na^+ in shoots for beetroot ($P < 0.001$) but not in maize. In beetroot, average Na^+ was about 281 cmol kg^{-1} at Lieu Tu and 145 cmol kg^{-1} at Long Phu (data not presented).

The K^+ concentrations between the crops were relatively similar between the two crops with ranges (5th to 9th percentile) of 32–68 cmol kg^{-1} for beetroot and 56–84 cmol kg^{-1} for maize. Site had also a significant effect on K^+ in shoots for beetroot ($P < 0.001$) but not in maize. In beetroot, average K^+ was about 59 cmol kg^{-1} at Lieu Tu and 37 cmol kg^{-1} at Long Phu (data not presented).

3.5 Relationships between soil conditions, ion concentrations in the shoots and yield (H4)

Our 4th hypothesis was that there would be links between yield, soil conditions and ion concentrations in shoots. The range of mulch treatments, sites and years experimented provided us with a range of conditions with which to test this. The complete range of relationships tested is summarized in the Supplementary Materials (Tables S7 and S8). The strongest relationships found (highest r^2 values, most significant *P*-values) are summarized in Fig. 7 (Part A for beetroot and Part B for maize).

3.5.1 Beetroot

For beetroot, the strongest relationships between soil conditions and yield were at soil depth 0-15 cm at the end of the year between: (a) solute potential and yield ($r^2 = 0.18$; $P = 0.003$), and (b) $EC_{1:5}$ and yield ($r^2 = 0.16$; $P = 0.004$) (Supplementary Materials, Table S8A). Most of the possible relationships between soil conditions and Na^+ in the leaves were significant (Supplementary

Materials, Table S9A). The strongest relationships ($P < 0.001$) were for soil at 0-15 cm sampled at the end of the growing season between solute potential and Na^+ ($r^2 = 0.64$; $P < 0.001$) and between $EC_{1:5}$ and Na^+ ($r^2 = 0.52$; $P < 0.001$). There was a negative relationship ($r^2 = 0.35$, $P < 0.001$) between yield (y-variable) and Na^+ in leaves (*x*-variable) (Fig. 7A).

3.5.2 Maize

For maize, most of the possible relationships between soil conditions and yield were significant (Supplementary Materials, Table S8B). The most important correlations were the negative relationships ($P < 0.001$) between $EC_{1:5}$ at 15-30 or at 30-40 cm with yield (r^2 values of 0.584 and 0.58, respectively). For maize, there was no positive relationship between $EC_{1:5}$ and Na^+ in the leaves (Supplementary Materials, Table S9B). There was a negative relationship ($r^2 = 0.13$; $P < 0.05$) between yield (*y*-variable) and Na^+ in leaves (*x*-variable) (Fig. 7B).

4 Discussion

This paper aimed to determine if the growth of alternative crops to rice was possible in the Mekong Delta, and to test four hypotheses. In overview, we can confirm that the growth of alternative crops is possible in the Mekong Delta: over two years of trials we produced commercial yields of beetroot and maize, but drought, salinity and, early season inundation of low-lying areas were significant risks. Regarding our hypotheses: (a) the use of rice straw mulches did increase crop yields (H1), (b) mulches decreased soil salinity, had no effect on soil water content, and increased soil solute potential (H2), (c) mulches decreased Na^+ in shoots (H3), and (d) there were relationships between soil conditions, ion concentrations in leaves and yield (H4).

4.1 Effects of mulch on yield, soil salinity, and soil water content (H1, H2)

4.1.1 Impacts on yield

In the present work, the use of rice straw as a mulch increased the yield of beetroot and maize by up to 114% and 48%, respectively. These gains in productivity, particularly for beetroot, are high compared with other examples from the literature. For example, rice straw mulches increased the yield of sunflower by 35% in moderately saline soils (Paul et al. 2020) and increased the yields of peanut, rapeseed, potato and onion by 27–69% in non-saline soils (Ramakrishna et al. 2006; Sarangi et al. 2010; Kar and Kumar 2007; Igbadun et al. 2012).

4.1.2 Impacts on soil

In the present work, the use of rice straw mulch decreased salinity in the upper soil profile (0-15 cm) by up to 13% but had no effect at deeper depths. This presumably occurred because mulch decreased the evaporation of water at the soil surface, which decreased the movement of salt to the soil surface by capillarity (H2). Use of rice straw mulch (5 t ha^{-1}) had a similar effect in southern Bangladesh, decreasing salinity in the upper soil profile (0-7 cm) by about 15% (Paul et al. 2020).

Contrary to our original expectations, mulch had no effect on soil water content. In our work, the water-table was ~20 cm beneath the soil surface (at the depth of the irrigation furrows) and soils were irrigated every day; any water lost from the upper profile would therefore have been immediately replaced through capillarity. In a contrasting study from southern Bangladesh, mulch increased the water content of the upper soil by 30% (Paul et al. 2020). However, here irrigation was only supplied on 3-5 occasions during the entire growing season, and the water-table would have been deeper (70-100 cm) (c.f. Islam et al. 2022).

4.1.3 Implications

Several practical factors emerge from these studies. Firstly, in our work the application of mulch at 10.5 t ha^{-1} had the same benefits (yield, soil salinity and Na^+ in the shoots) as a mulch application of 7 t ha^{-1}. In southern Bangladesh the application of rice straw mulch at 10 t ha^{-1} had the same benefit to sunflower yield as a mulch application of 5 t ha^{-1} (Paul 2020). It appears that mulch applications of 5-7 t ha^{-1} are therefore adequate for salinity abatement. Secondly, the effects of mulch on soil water content appear to be mediated by factors affecting the availability of water at the site (e.g. frequency of irrigation, depth to water-table). Our work suggests that there will be little benefit from mulches in non-saline soils where water-tables are shallow or where irrigation is frequently applied. However, mulching can reduce water evaporation, improve fallow efficiency, and reduce salt build-up in the soil (Pang et al., 2010; Li et al., 2013; Zhao et al., 2014). It would be useful to test these two conclusions in a wider range of environments (salinities and water availability), with a wider range of crops of differing salt tolerance. In addition, future research should quantify the economic threshold of mulch application across a salinity gradient (ECe) to optimize resource allocation for smallholder farmers.

One final important researchable question remains. Agriculture in many developing countries is becoming increasingly challenged by the increasing expense of fertilizers. We wonder whether the use of surface soil mulches could change the accessibility of nutrients to crops. There is now increasing evidence that crops preferentially access water from the least saline parts of their root system (see recent review by Valenzuela et al. 2022). If mulches decrease the salinity of surface soils, then the question arises: do they also increase the accessibility of nutrients in the surface soil to crops? We are not aware of any research that has been conducted in this area.

4.2 Feasibility of alternative crops – relative adaptation of beetroot and maize

With $EC_{1:5}$ values ranging from 0.2 to 0.8 dS m^{-1} at 0–15 cm, and silty clay soil textures, the surface soils of Long Phu and Lieu Tu would have had EC_e values in the range 1.8–7.1 dS m^{-1} (calculated based on the $EC_{1:5}$ to EC_e conversion factors of Slavich and Petterson 1993) and therefore varied from being non-saline to moderately saline (Rogers et al. 2005).

One of the most impressive results from our study was the exceptional level of production achieved with beetroot (average yield for the highest mulch treatment = 42 t ha^{-1}), compared with maize (average yield for the highest mulch treatment = 5.4 t ha^{-1}). The differences in productivity between these two crops are of agricultural interest and reflect the fact that beetroot is a halophyte, whereas maize is a glycophyte.

Halophytes adapt to salinity by taking up and using Na^+ for osmotic adjustment and compartmentalizing this in the vacuole (Flowers and Colmer 2008). The substantially higher concentrations of Na^+ in the shoots of beetroot (132–351 cmol kg^{-1}) compared with maize (15–39 cmol kg^{-1}) reflect its halophytic origin. Concentrations of a similar magnitude have been found in other studies. For example, average Na^+ concentrations were 311–335 cmol kg^{-1} in leaves of young beetroot plants grown with 55 mM NaCl under controlled conditions (Subbarao et al 2001).

Given, its halophytic origin, we might expect beetroot growth to respond positively to the presence of some salt in the soil. This has been confirmed in multi-year experiments conducted in Russia on a non-saline soil in which plants were fertilized with combinations of NPK and low levels of NaCl (Ubugunov et al. 2021). Compared to unfertilized controls, the use of NPK alone increased the average yields of beetroot roots and shoots by 38% and 69%, respectively. However, the application of NPK plus NaCl (40 kg ha^{-1}) increased average yields of roots and shoots by 68% and 123%, respectively (Ubugunov et al. 2021). At higher concentrations of salinity beetroot has decreased growth, but these decreases are less severe than for salt sensitive species. For example, in comparisons of the responses to salt of beetroot and carrot (a salt sensitive root crop), salinity (-0.4 MPa) decreased the fresh mass of beetroot root by 34% but decreased the fresh mass of carrot root by 83% (Magistad et al. 1943). In another trial, salinity (-0.4 MPa) decreased total plant fresh weight by 25% in beetroot but by 96% in carrot (Bernstein et al. 1974).

Do the relationships between beetroot yield and soil salinity in Fig. 7 duplicate results from the literature? Our lines of best fit between $EC_{1:5}$ and yield for beetroot (Fig. 7A) and for maize (Fig. 7B), suggest a 50% decrease in relative yield at an $EC_{1:5}$ of ~0.6–0.7 dS m^{-1}, equivalent to EC_e values of 5–6 dS m^{-1} (calculated based on the conversion factor of Slavich and Petterson 1993 for soils of this texture). Previous published studies suggest that under well-watered conditions beetroot and maize can be expected to have 50% decreases in relative yield at EC_e values of ~9.2 and ~5.5 dS m^{-1}, respectively (Steppuhn et al. 2005).

4.3 On rooting depth and the vulnerability of crops

Although rooting depth was not directly measured in our trials, indirect evidence suggests that waterlogging affected the patterns of rooting in the crops, which affected the kinds of relationships that occurred between salinity and yield, and yield and mulch. The elements of this argument are as follows.

4.3.1 Presence of waterlogging

Irrigation was supplied to the plots daily, and seepage from the irrigation furrows would have ensured that the depth to water-table in the field was generally within ~20 cm of the soil surface. Capillarity would have ensured that the zone of soil hypoxia extended for ~10 cm above the water-table (Wesseling and van Wijk 1957). We therefore believe that the crops only had about 10 cm of well-aerated soil in which to grow. There is now a substantial body of evidence from other studies which suggests that damage due to waterlogging can occur in annual crops as the depth to the water table becomes shallower than 30 cm. Researchers quantify the severity of waterlogging by calculating the sum of excess water above 30 cm depth (SEW30) (Sieben 1964; Bennett et al. 2009; Islam et al. 2022). One way to decrease the risk of waterlogging may be to hydrologically isolate fields using elevated bunds around fields and planting crops on raised beds. This approach has improved the sustainability of crop production in landscapes at risk of waterlogging in Asia, Australia, and Mexico (Roth et al. 2005). We are not aware of the use of shallow surface drains or raised beds having been previously attempted for growing alternative crops in Vietnam.

4.3.2 Crop tolerance

As noted in the Introduction, the key physiological adaptation that delivers plant tolerance to waterlogging is the development of aerenchyma in roots, which maize forms (Drew et al. 1980) but beetroot cannot (Rojas-Méndez et al. 2021). The presence of this adaptation would have enabled maize roots to penetrate the hypoxic soil below the shallow water-table, but non-aerenchymatous beetroot would not have been able to do this.

4.3.3 Responses of yield to salinity

One of the impressive features of our study was that for maize there were many significant correlations (5 at $P < 0.001$; 6 at $P = 0.017$; 7 at $P = 0.05$) between crop yield and $EC_{1:5}$ (or Ψ_s) (Table S8 Supplementary Materials). The fact that these relationships occurred across all soil depths suggests that rooting depth in maize was not constrained at the two sites. By contrast, with beetroot, there was only 1 significant relationship ($P = 0.009$) between yield and $EC_{1:5}$ or Ψ_s ($P < 0.01$) and this was at 0-15 cm (Table S8, Supplementary Materials). The irrelevance of deeper $EC_{1:5}$ or Ψ_s values to the yield of beetroot suggests that these plants may have been shallow-rooted.

4.3.4 Responses of yield to mulch

In the present work, soils had higher salt concentrations in the upper soil (0-15 cm) than deeper soil layers (15-40 cm), but it was only in the shallow soil that mulches deceased salinity (Fig. 5). In beetroot (the crop which we argue was shallow rooted) mulches increased crop yield by more than 100%. By contrast, in maize (the crop which we argue had roots in both shallow and deeper soil), mulches only increased yield by ~50% (Fig. 3).

5. Conclusion

This paper provides an informative case study in saline agriculture. The study assesses the suitability of alternative crops to rice and management techniques to diversify income for people in vulnerable areas with the climate change. Here we show for the first time that exceptional yields are possible on salt-affected delta soils if attention is focused on the key points of vulnerability. Commercial crop yields of the scale of 42 t ha^{-1} are rare in saline agriculture. We achieved these yields because of a combination of factors. Beetroot is a halophyte and is adapted to the salinity of these sites. It was watered regularly (daily in most cases) and was grown with a soil surface mulch that decreased surface salinity. By contrast, yields were low with the non-halophytic crop (maize) and were lower without the mulch. Furthermore, The two studied sites had adequate internal drainage, and therefore, there was no waterlogging. Climatic conditions in the Mekong Delta during the trial period were favorable for beetroot production.

The trials at the two more elevated sites were successful, but that at the less elevated site failed because high rainfall and inadequate internal drainage prevented sowing. This result shows that inundation and waterlogging are potential hazards in the Mekong Delta. It may be possible to grow alternative crops to rice in low-lying areas of the Mekong Delta, but this will probably require other interventions to decrease inundation/waterlogging risk such as the implementation of shallow surface drains or the use of raised beds.

In overview, saline agriculture has been researched since the 1970s, but the number of successful industries based on the growth of better crops for saltland is still relatively small. Widespread adoption requires not only the selection of the right plant but also the development of the associated supporting technologies and the market chains that lead to financial gain for participating farmers. The journey of turning beetroot into a viable saline agricultural industry for Vietnam will require many further steps where technical advancements must be integrated within robust socio-economic frameworks.

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Authors' contributions Conceptualization, DDM, CMK, JC, BK, SO and EGBL; methodology, JC, EGBL, DDM and CMK; data curation, DDM, CMK, TDK, NTKP, CDAG, DTTL and TBL; investigation, DDM, CMK, TDK, NTKP, CDAG, DTTL and TBL; writing (original draft), EGBL and DDM; writing (review and editing), EGBL, DDM, BK and JC; funding acquisition, JC, CMK, EGBL and SO; resources, CMK and DDM; supervision, DDM, CMK and JC. All authors were involved in the revision of the draft manuscript and have approved the final content.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable

Declarations

Ethics approval Not applicable

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Paper 4: Effects of rice straw mulching on nematode community in upland-paddy rice systems in salt-affected soils

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Abstract. Rice straw mulching is a biomass utilization practice for soil management that influences soil microbial communities. However, its effects on nematodes community under upland-rice systems in salt-affected soils remain unclear. This study examines nematode community responses to rice straw mulching at 0, 3.5, 7.0, and 10.5 tons ha^{-1} in paddy fields, with no mulching as a control in two distance sites in the Mekong Delta, Vietnam. A total of 35 and 37 nematode genus were identified at Long Phu and Lieu Tu, respectively, with bacterivores as the dominant group, followed by herbivores. *Acrobeloides, Hirschmanniella, Chronogaster, Aporcelaimellus,* and *Prismatolaimus* were prevalent in Long Phu, while *Acrobeloides, Prismatolaimus, Hirschmanniella*, and *Alaimus* dominated in Lieu Tu. Nematode community structure varied due to soil properties difference among sites. The highest mulching rate (10.5 tons ha^{-1}) increased total nematode abundance, particularly cp1, cp2, and cp5 groups, while reducing plant-parasitic nematodes. It also enhanced total nematode biomass and metabolic footprints, indicating improved soil fertility. At Long Phu, mulching increased biodiversity, as reflected in higher species richness and Shannon-Wiener indices. Moreover, the abundance of plant-parasitic nematodes decreased under the highest mulching rate. These findings suggest that applying 10.5 tons ha^{-1} of rice straw mulch promotes soil structure and nematode diversity, supporting agricultural sustainability in salt-affected, arid soils impacted by climate change.

Key words: fertility, free-living nematodes, soil organisms, crop rotation, straw mulching.

1. Introduction

Rice is an important crop in Vietnam, particularly in the Mekong Delta (MD) region, Southern Vietnam which is cultivated on 82% of the country's total agricultural land (Vu et al., 2018) and accounts for 22% of the nation's Gross Domestic Product (GDP) and making up 45% of Vietnam's total rice-growing area and producing 57% of the nation's total rice output (Tong, 2017). The annual cultivation of three rice crops is an extremely intensive cropping pattern in this area and it has been practiced for long-time ago. To maintain productivity, the overuse of chemical fertilizers and pesticides are concerned (Tran et al., 2018) on water pollution (Stone and Hornberger, 2016), emissions (Hoa et al., 2018), pests and diseases resistance (Stuart et al., 2014), and soil degradation (Cassman and Harwood, 1995). Moreover, the MD is facing with serious difficulty due to climate changes such as drought conditions and salinity intrusion (Tran et al., 2019; Wassmann et al., 2019). Previous studies reported that saltwater intrusion has significantly negative effects on soil properties and crop productivity due to the accumulation of water-soluble salts (Kruse et al., 2020; Truc et al., 2020; Wang, T. Y. et al., 2019), and had negative effects on soil-dwelling nematode communities such as their composition, trophic structure and diversity (Sinh et al., 2021; Wang, J. Q. et al., 2019; Zhao et al., 2021).

Soil nematodes are a diverse group of microscopic animals (0.3-5.0 mm in length) that can be classified into 'functional groups' based on their morphological characteristics and feeding behavior (Ferris et al., 2001b). Free-living nematodes (FLN) include bacterivores, fungivores, omnivores, and predators, which feed on a variety of soil organisms and organic matter such as bacteria, fungi, other nematodes, or small soil invertebrates, play an important role in the soil food web by promoting organic matter decomposition, nutrient cycling, and improving soil fertility (Buchan et al., 2013). In contrast, plant-parasitic nematodes (PPN) are among the most significant pests in paddy rice, contributing to an annual global yield reduction of 10-20% (Nicol et al., 2011). For example, species of the genus *Meloidogyne* can reduce rice productivity by up to 80% (Soriano et al., 2000). In the VMD, *Hirschmanniella oryzae* is another species found in irrigated paddy rice (Sinh et al., 2021) and its distribution has been reported in many Asian countries (Bridge et al., 2005).

Agricultural practices have various effects on soil organisms, particularly the composition of the nematode community and their ecological indices. For instance, straw mulching types (rice, rapeseed, and their mix) with an amount of 3 kg/m^2 (equivalent: 30 tons/ha) and straw mulching coverage levels (covering the mean radius of the crown width, covering 1.5 times the mean radius of the crown width and covering the whole experimental plot) in walnut orchard field in Langzhong, central Sichuan Basin, southwestern China. This study reported that the mulching reduced the total number of nematodes but enhances the metabolic footprint of higher trophic levels within the nematode community (Song et al., 2020). The application of organic matter significantly promotes soil nematodes, Luo et al. (2021) explored the effects of residue mulching on belowground nematodes, especially focusing on the production and respiration of fungivores. Moreover, the application of both raw and composted residues also influences the nematode community (Li et al., 2018; Nahar et al., 2006), with belowground nematode populations being significantly driven by aboveground plant biomass (Yeates, 1999). Nematodes can be used to assess soil health, with those belonging to functional guilds cp4 and cp5 serving as indicators of soil stability and maturity due to their sensitivity, larger body size, and slower reproductive cycles (Du Preez et al., 2018; Ekschmitt et al., 2001) and they are commonly used to assess the effects of salinity (Su et al., 2012) or environmental changes (de Goede, 1993) because of their sensitivity to microhabitats (Tsiafouli et al., 2017). Rice straw mulching has beneficial effects on soil water content conservation, salinity reduction, and microbial activity (Kaveney et al., 2023).

The changes that upland crops from continuous rice production show positive effects on promoting the beneficial nematodes community and as a systems that can reduce the infestation of plant-parasitic nematodes (Liu, Ting et al., 2016). Sinh et al. (2020) reported that the use of sesame and soybean in continuous rice enhanced the beneficial nematodes community composition and reduced the number of *Hirschmanniella* in soils. This consistence is also observed in the study by Win (2011) who reported that the changes in chickpea and blackgram in rice paddy field reduced the abundance and number of galls infected by *Meloidogyne graminicola*. The nematode community composition changes in alfalfa and maize intercropping, indicating the promotes beneficial nematodes in intercropping system (Teshita et al., 2023). These changes from flooded paddy rice to upland conditions in a short period may be key factors influencing the nematode community, as they help provide suitable microhabitats for nematodes and other soil organisms. Previous studies reported that the application of straw mulching enhances soil biological, chemical properties and changes the roots distribution in upland conditions. For instance, straw mulching is considered is a soil management practice for sustainability in agriculture that enhanced water availability in soybean fields (Li et al., 2022), increased roots occurrence of maize (Thidar et al., 2020) and promoted microbial community structure and functions in the maize field (Liu et al., 2023). Therefore, we hypothesis that rice straw mulching may promote nematodes community composition in relation to water availability in soils, particularly in salt-affected soils where water is most limiting factors. Thus, this study aims to examine changes in nematode community composition, particularly in trophic structure, metabolic footprint, and diversity under different mulching ratios in climate affected regions of the MD.

2. Materials and methods

2.1. Experiment designs

A field trial was conducted in two different sites at Long Phu and Lieu Tu, Soc Trang province (Fig. 1). The soil type is classified as Salic Fluvisols at Long Phu and the main soil texture of Lieu Tu and Long Phu is silty clay (Soil Survey Staff, 1998). This paddy field had traditionally practiced a triple rice cultivation system (three crops of rice per year) until the past 3 years where upland crops were introduced during the dry season. Details in crop timing are shown below (Fig. 2). Initial soil properties were analysed and reported in Table 1.

Fig. 1. The field experiment layout at Lieu Tu and Long Phu site

Table 1: Initial soil properties at two field trials in Lieu Tu and Long Phu site

Randomized block design were placed in the paddy rice fields in Long Phu and Lieu Tu. The mulching application rate was no mulching (M0), 3.5 tons ha^{-1} (M1), 7.0 tons ha^{-1} (M2) and 10.5 tons ha^{-1} (M3). Five treatments were set up with three replicates each field trial. Cowpea (*Vigna unguiculata*) was sown in the dry season in February 2023 with the different mulching rates applied (M0, M1, M2, M3), and a fallow (without mulching, no crop) treatment was used as a reference control.

2.2. Sampling and analysis

2.2.1. Soil sampling

Soil samples were collected at the beginning of the cowpea crop in February, 2023 (rice was previous crop) and at the end of cowpea season in May 2023. This is corresponding to 2 years field experiment in two fields (Fig. 2). Soils were collected from five points, responding to five subsoil samples on each plot by using an auger from 0 cm to 20 cm depth. Then, five subsoil samples from each plot were mixed evenly for nematode extraction.

Fig. 2. Cropping seasons in triple-rice intensive systems in the VMD (A) and sampling point (B), adapted by Kaveney (2023). First sampling (February, 2023) is beginning of cowpea crop (rice was previous crop), second sampling (May, 2024) is end of cowpea crop. **Note:** in Fig. A, the orange color indicates the dry season in the VMD and the blue color indicates the duration of upland crops.

2.2.2. Nematode extraction

A subsample (20 g) of homogenous moist soil from each plot was used for nematode extraction using the Baermann's funnel method (van Bezooijen, 2006)**.** Nematodes suspensions were collected after 48 hour at room temperature. Hot formaldehyde 4% solution was used to kill nematodes and stored. A few drops of 1% rose Bengal solution were added to dye the nematodes. The total numbers of individual nematodes were counted under a microscope and converted to the density per 100 g of dry soil. Nematodes were mounted in a drop of concentrated glycerin (99.5%) on a glass slide and sealed with a paraffin ring for nematode identification. All nematodes were assigned to five trophic groups; bacterivore, fungivore, PPN, omnivore and predator (Yeates et al., 1993). The classification of nematode colonizer-persister (c-p) value was based on life history strategies (Bongers, 1990; Bongers and Bongers, 1998)**.** For example, nematodes of cp1 have short generation time, high fecundity, and are mainly bacterivorous that feed on enriched media. Nematodes of cp2, cp3, and cp4 have longer generation times, greater sensitivity to adverse conditions and soil disturbance, and are mainly bacterivorous, fungivorous, predator, and small omnivorous. Nematodes of cp5 have the longest generation time, largest body, lowest fecundity, greatest sensitivity to soil disturbance, and are mainly omnivorous and predator (Ferris et al., 2001a). The maturity index (MI), plant parasitic index (PPI) and the ratio of total bacterivore and fungivore to the total PPN were calculated (Yeates et al., 1993). The number of genera (S), density (N), H' Shannon-Weiner diversity, species richness Margalef (d), evenness index (J), Simpson index (λ) and Hill's (N1, N2) index, were calculated using a PRIMER package version 6 (Clarke and Gorley, 2006). Metabolic footprints, community indices such as total maturity index (∑MI) and plant-parasitic index (PPI) were calculated using the NINJA online program at https://sieriebriennikov.shinyapps.io/ninja/(Ferris, 2010; Sieriebriennikov et al., 2014). Nematode metabolic footprints quantify carbon utilization by different food web components and provide information of energy flow through various trophic groups, which gives additional descriptive information on food web form and soil functions (Ciobanu et al., 2015; Ferris, 2010; Ferris et al., 2001a; Sanchez-Moreno et al., 2009). For example, the enrichment footprint is the metabolic footprint of nematodes, which rapidly responds to the resource enrichment. The structure footprint is the metabolic footprint of higher trophic levels, which may have a regulatory function in the food web and which are indicative of the abundance of organisms of similar functions in non–nematode taxa. The herbivorous, bacterivorous, omnivorous, fungivorous and predator footprints are based on the nematode indicators of carbon and energy entering the soil food web through their respective channels.

2.3. Data analysis

Data were analyzed using one-way ANOVA to determine the effects of rice straw mulching on nematode community composition and their ecological indices. Nematode abundances were log-transformed Log (X+1) prior to statistical analysis to assess data normality and to test for homogeneity of variances using Levene's test. Post hoc tests were applied to compare significant differences among treatments using Tukey's HSD test. Differences with a p-value < 0.05 were considered statistically significant. Additionally, principal component analysis (PCA) was used to demonstrate the distribution patterns of taxonomic nematode communities mulching application rates and to identify the main variables corresponding to soil. The PCA was conducted on a full dataset of normalized nematode abundance by using PRIMER version 6 (Clarke and Gorley, 2006).

3. Results

3.1. Rice straw mulching affected nematode community composition

3.1.1. Nematode abundance

The application of rice straw mulching did not affect the total abundance of the nematode community in soils at the Lieu Tu site, but it tended to increase the abundance at M3 (10.5 tons ha^{-1}) (Fig. 3A). In Long Phu, the abundance of nematodes increased with higher mulching rates and was significantly higher ($p < 0.05$) at M3 compared to the fallow treatment at the end of the cowpea season (Fig. 3B).

Fig. 3. Abundance (mean±SE, n=3) under rice straw mulching at Lieu Tu (A) and Long Phu (B) field trials, Soc Trang province. Different letters indicate the significant differences among treatments at $p < 0.05$ by Tukey HSD test.

3.1.2. Taxonomic composition of nematodes

In Lieu Tu, a total of 37 nematode genera were recorded, including 33 genera at the beginning and 37 genera at the end of the cowpea season, respectively. In Long Phu, a total of 35 genera were identified, including 31 genera at the beginning and 35 genera at the end of the cowpea season. The number of genera did not differ among the rice straw mulching treatments in Lieu Tu at both sampling times (Fig. 4A). In Long Phu, the number of genera with a mulching rate of 10.5 tons ha^{-1} was significantly greater ($p < 0.05$) than in the fallow and no mulching treatments (Fig. 4B).

Fig. 4. Number of genera (mean±SE, n=3) under rice straw mulching at Lieu Tu (A) and Long Phu (B) field trials, Soc Trang province. Different letters indicate the significant differences among treatments at $p < 0.05$ by Tukey HSD test.

The most abundant genus at the Lieu Tu site at the beginning of the cowpea crop season was *Acrobeloides*, accounting for 23.1%, followed by *Hirschmanniella* (21.3%) and *Mesodorylaimus* (5.1%). At the end of the crop, the most abundant genera were *Acrobeloides* (23.2%), *Mesorhabditis* (15.0%), *Hirschmanniella* (11.3%), and *Aphelenchoides* (5.9%) (Fig. 5A, B). At the Long Phu site, the most dominant genus at the beginning of the season was *Acrobeloides* (16.7%), followed by *Hirschmanniella* (12.7%), *Chronogaster* (9.1%), *Aporcelaimus* (4.9%), *Prismatolaimus* (4.5%), and *Mesodorylaimus* (4.5%). At the end of the crop, the dominant genera were *Acrobeloides* (23.6%), *Hirschmanniella* (12.9%), *Aphelenchoides* (5.3%), and *Mesorhabditis* (4.1%) (Fig. 5C, D).

Fig. 5. Proportion (n=15) of each genera in 100g dry soil at the beginning and the end of cowpea crop in Lieu Tu (A, B) and Long Phu (C, D), respectively.

In Lieu Tu, the density of *Aporcelaimus* was significantly greater ($p < 0.05$) in the M3 treatment compared to the M1 and M2 treatments at the beginning of the season. *Mylonchulus* was greater abundant ($p < 0.01$) in M2 compared to the fallow, M1, and M3 treatments. *Thornenema* was more prevalent ($p < 0.05$) in M3 than in M0, while *Tylenchorhynchus* was more abundant ($p < 0.01$) in M0, M1, and M3 than in fallow. The density of *Tylenchus* was higher ($p = 0.03$) in M3 compared to M1 (Table S1). At the end of the crop season, *Acrobeloides* was more abundant ($p < 0.05$) in M3 than in M1, and *Amphidelus* was more abundant ($p < 0.05$) in M3 than in M0 and M2 treatments. *Aphelenchoides* was more abundant ($p = 0.007$) in M2 and M3 than in fallow, and more abundant in M3 than in M1. *Aporcelaimus* was more abundant ($p < 0.05$) in M3 than in M1. *Mononchus* was significantly more abundant ($p = 0.001$) in M2 and M3 than in fallow and M0 treatments (Table S2).

In Long Phu, the density of the bacterivore genus *Mesorhabditis* was greater ($p < 0.01$) in M3 than in M0, M1, and M2 treatments. The density of *Panagrolaimus* was lower ($p < 0.05$) in M2 than in fallow and M3 treatments at the beginning of the crop season (Table S3). At the end of the crop season, the density of the bacterivore genus *Acrobeloides* was greater ($p < 0.05$) in M3 than in fallow, and *Diploscapter* was more abundant ($p < 0.05$) in M3 than in fallow and M1 treatments. *Panagrolaimus* was more abundant ($p < 0.05$) in M2 than in fallow and M1 treatments (Table S4).

3.2. Trophic structures of nematodes community changes under rice straw mulching

In Lieu Tu, there were no differences among mulching application rates in the proportions of plant-parasitic nematodes (PPN), bacterivores (Ba), fungivores (Fu), omnivores (Om), and predators (Pre) in soils at the beginning of the season. However, there was a significant difference in omnivores ($p < 0.05$) between the M2 and fallow treatments, and the percentage of PPN in M3 was significantly lower ($p < 0.05$) than in the fallow treatment (Fig. 6A).

In Long Phu, a difference ($p < 0.05$) was observed in the percentage of PPN between the M3 and fallow treatments, with the percentage of PPN in M3 being lower than in the fallow treatment (Fig. 6B).

Fig. 6. Proportion of each trophic structure (mean, n = 3) in soils at the beginning and end of the cowpea cropping season in Lieu Tu (A) and Long Phu (B). Different letters indicate significant differences among treatments at $p < 0.05$, as determined by the Tukey HSD test. Trophic structures include omnivores (Om), predators (Pre), bacterivores (Ba), fungivores (Fu), and plant-parasitic nematodes (PPN).

3.3. Functional-guilds of free-living nematodes community changes under rice straw mulching

In Lieu Tu, there were no differences among mulching application rates in the density of cp1, cp2, and cp3 groups in soils at the beginning of the season. However, cp4 density was significantly higher ($p < 0.05$) in M3 compared to M0, M1, and M2 treatments. The abundance of cp5 was also significantly greater ($p < 0.05$) in M3 than in M0 (Fig. 7A). By the end of the season, cp2 abundance was significantly greater ($p < 0.01$) in M3 than in M1 and fallow treatments. In Long Phu, cp1 and cp2 abundances were significantly greater in M3 than in M1 and fallow treatments at the end of the season (Fig. 7B).

Fig. 7. Abundance of each functional guilds, cp (colonizer-persister) (mean±SE, n = 3) in soils at the beginning and end of the cowpea cropping season in Lieu Tu (A) and Long Phu (B). Different letters indicate significant differences among treatments at $p < 0.05$, as determined by the Tukey HSD test. **Notes:** nematodes of cp1 have short generation time, high fecundity, and are mainly bacterivorous that feed on enriched media. Nematodes of cp2, cp3, and cp4 have longer generation times, greater sensitivity to adverse conditions and soil disturbance, and are mainly bacterivorous, fungivorous, predator, and small omnivorous. Nematodes of cp5 have the longest generation time, largest body, lowest fecundity, greatest sensitivity to soil disturbance, and are mainly omnivorous and predator (Ferris et al., 2001a).

3.4. Effects of rice straw mulching on total biomass and metabolic footprints of nematodes community

The biomass of nematodes varied among treatments. In Lieu Tu, the total biomass of nematodes was significantly greater ($p < 0.05$) in the M3 treatment compared to M2 in soil at the beginning of the season. By the end of the season, total biomass was significantly higher in M3 than in the fallow, M0, and M1 treatments (Fig. 8). In Long Phu, the total biomass was significantly higher in M3 than in M0 at the beginning of the season, and it tended to increase in soil by the end of the season, corresponding to a greater amount of mulching, but it is not significant.

Fig. 8. The biomass of nematodes (mean±SE, n = 3) in soils from Lieu Tu (A) and Long Phu (B). Different lowercase and uppercase letters indicate significant differences among treatments at $p < 0.05$ at the beginning and end of the season, respectively, within each trial, as determined by the Tukey HSD test.

In the Lieu Tu trial, the composite footprint ($p = 0.008$), structure footprint ($p = 0.019$), and omnivore footprint ($p = 0.028$) were significantly greater in M3 than in M0, M1, and M2 treatments in soils at the beginning of the season (Table 2). The composite footprint was significantly greater ($p = 0.007$) in M3 than in the M1 and fallow treatments at the end of the season. The structure footprint, bacterivore footprint, and omnivore footprint in M3 were significantly greater ($p < 0.05$) than in the M1 treatment at the end of the season.

In the Long Phu trial, the composite footprint, structure footprint, bacterivore footprint, and omnivore footprint were highest in M3 compared to other treatments at the beginning of the season, but the differences were not significant. At the end of the season, the composite footprint ($p = 0.05$) and bacterivore footprint ($p = 0.019$) were significantly higher in M3 compared to the fallow treatment, and the enrichment footprint ($p = 0.016$) in M3 was greater than in both the fallow and M0 treatments.

Table 2. Metabolic footprints (mean±SE, n = 3) of nematodes community among treatments in Lieu Tu and Long Phu trial. Different letters indicate significant differences among treatments, as determined by the Tukey HSD test. ns-not significant.

3.5. Ecological index of nematodes community changes by the mulching effects

In Lieu Tu, there were no significant differences in biodiversity indices such as species richness (d) and the Shannon-Wiener index (H′) among mulching treatments, though these indices tended to increase in soils with higher amounts of rice straw mulching (Fig. 9A). In Long Phu, significantly higher values of species richness ($p < 0.05$) and the Shannon-Wiener index H′ ($p < 0.05$) were observed in soils under M2 and M3 treatments compared to the fallow treatment at the end of the season (Fig. 9B).

Fig. 9. Diversity indices of the nematode community (mean, n = 3) in soils from Lieu Tu (A) and Long Phu (B). Different lowercase and uppercase letters indicate significant differences among treatments at $p < 0.05$ at the beginning and end of the season, respectively, within each trial, as determined by the Tukey HSD test.

4. Discussion

4.1. Rice straw mulching induces changes in nematode community composition

Principal component analysis showed that nematode community composition varied between the two field trials (Fig. 10). The differences in nematode community composition between locations can be explained by soil types and properties, which are primary factors affecting soil organisms, including nematodes (Kitagami and Matsuda, 2024) and climate condition effects (Ma et al., 2018; Wang, J. Q. et al., 2019).

Fig. 10. Principal component analysis based on the community of nematodes in Long Phu and Lieu Tu.

In our study, soil properties differed between the two sites in terms of electrical conductivity (EC) and cation content (Table 1), particularly with notable differences in soil organic carbon. Our previous study reported that salinity intrusion, which increases soil EC and the amount of soluble sodium, led to changes in the belowground nematode community (Sinh et al., 2021), and soil pH is also a related factor (Burns, 1971). Soils rich in organic matter provide abundant food resources for bacterivores and fungivores, thus influencing the abundance of these groups. Organic carbon is well known to be a critical factor affecting nematode communities in soil (Quist et al., 2019) , as it directly and indirectly affects their food sources, habitat, and overall soil health (Giesselmann et al., 2019). The **fertilization and soil management are also induces changes in soil properties (Birkhofer et al., 2008;**

Roth et al., 2015) that drive the nematodes and other soils organisms community composition (Mills et al., 2020). For instance, organic amendments like rice straw mulching increase organic matter and microbial biomass (Luo et al., 2021; Song et al., 2020), supporting bacterivores and promoting a more diverse nematode community. Chemical fertilizers might support fewer nematode groups by altering pH or creating imbalances in microbial communities or it changes the environmental conditions in soil habitats (Hoa et al., 2018; Neher, 1999). Previous study reported that the growth of leguminous plants promotes nematodes community composition and enhance the soil available nitrogen (Teshita et al., 2023). The beneficial nematodes in paddy field changes rapidly under upland-paddy rice rotation which responded to the changes in soil properties that the nitrogen availability and microbial activity were major effects (Djigal et al., 2004; Sinh et al., 2020; Sun et al., 2013).

The abundance of the nematode community was increased at the highest rate of mulching at 10.5 tons ha^{-1}, representing the increasing of bacterivore, fungivore and omnivore groups, while it reduced the abundance of plant-parasitic nematodes. Our results can be explained by the higher mulching application rates providing more residues to microbial activity where bacteria and fungi are the main community involved. Liu et al. (2016) reported that the greater abundance of bacterivore genus *Acrobeloides* and fungivore genus *Filenchus* were recorded in upland conditions. In our study, the fungivore genus *Aphelenchoides* was dominant at the end season. This observation agrees with Sohlenius (1985) and Okada et al. (2011), who reported that a lower density of *Aphelenchoides* was observed under wetter conditions.

4.2. Trophic structure and functional guilds of nematodes community responded to rice straw mulching

Rice straw mulching induced changes in the nematode trophic structure in our study, especially the omnivore and plant-parasitic nematodes groups. In which, the mulching increased abundance of omnivore, whilst reducing the abundance of plant-parasitic nematodes in soils. Based on the functional guilds of nematodes, resulted showed that the abundance of cp1, cp2 and cp4 were increased according to higher amount of rice straw mulching. Straw mulching induces changes in soil conditions like water contents, temperature and further support the composition and activity of soil microbial communities, including bacteria and fungi, play a major role in shaping nematode communities (Birkhofer et al., 2008; Klusmann et al., 2022). Bacterivorous nematodes, for instance, thrive in soils with high bacterial populations, while fungivorous nematodes are more prevalent in soils with abundant fungal networks. High organic matter also enhances soil structure, which can support a broader range of nematode trophic groups. Soil pH and nutrient availability, particularly nitrogen and carbon levels, directly influence the types of bacteria and fungi present, indirectly shaping nematode communities, as different nematodes prefer specific microbial populations. Nematodes are highly sensitive to soil moisture and temperature (Bakonyi and Nagy, 2000; Briar et al., 2012) and they responded to climate and plant resource type (Zhao et al., 2021). Moist soils provide an ideal environment for nematode movement and feeding, whereas drought can reduce nematode populations or limit them to specific groups adapted to low moisture. In a paddy rice field, soil organic matter tends to accumulate during the long-term submergence conditions, compared to aerobic conditions (Xuan et al., 2012). Higher soil organic matter content could increase bacterivore feeders in upland crops. For instance, the greater abundance of microbial communities increases the abundance of bacterivore and fungivore nematodes in upland soils (Liu et al., 2008; Zhong et al., 2016). Okada et al. (2011) also reported that the greater bacterivore and fungivore nematodes were observed in upland crop fields rather than in paddy rice fields. A greater abundance of bacterivores, fungivores, omnivores and predators was observed in hairy vetch or rye than in fallow conditions (Ito et al., 2015). Thereby, our results suggest that mulching application can be a feasible practice to increase the soil microbial community and numbers of bacterivors and fungivore nematodes, and may enhance crop productivity leading to a sustainable agriculture practice in the future.

4.3. Effects of rice straw mulching on metabolic footprints and ecological indices

The metabolic footprints of bacterivorous and omnivorous nematodes were greatest in M3 (10.5 tons ha^{-1}) compared to fallow or M0 (without mulching) treatments. This can be explained by the fact that mulching enhances microbial activity and other soil physicochemical properties (Linh et al., 2016; Nguyen et al., 2020), which likely increases food resources for nematodes across different trophic levels in the soil habitat. Additionally the application of mulching can stimulate greater food resources both above and belowground, it can also reduce the water loss that potentially supports higher trophic functional guilds such as omnivores and predators due to prey-predator interactions in the soil food web (Ferris, 2010). Previous studies have reported that the application of compost has been shown to enhance the abundance of bacterivorous nematodes in several crops (Thoden et al., 2011), taking rice straw mulching a suitable resource that supplies energy for microbial activity. Zhong and Zeng (2019) also reported that the application of organic amendments increased the biomass and activity of bacterial communities in soil. Thoden et al. (2011) found that the number of FLN, especially bacterivorous and fungivorous nematodes, typically increases significantly following the addition of any form of organic soil amendment.

5. Conclusions

Rice straw mulching induced changes in nematode community composition. A mulching rate of 10.5 tons ha^{-1} is recommended as a practice that stimulates the trophic structure of the nematode community, particularly at higher trophic levels, such as omnivores and predatory nematodes. This suggests that the soil conditions are mature, stable, and fertile. Mulching at 10.5 tons ha^{-1} significantly increased the total biomass, composite footprint, structure footprint, and predator footprint of the nematode community, reflecting soil maturity, fertility, and a moderate C/N ratio. Our results indicate that the application of mulching at 10.5 tons ha^{-1} could enhance soil biological properties by promoting nematodes at higher trophic levels. These findings suggest that mulching practices can be beneficial for agricultural sustainability, particularly in arid soils impacted by climate change.

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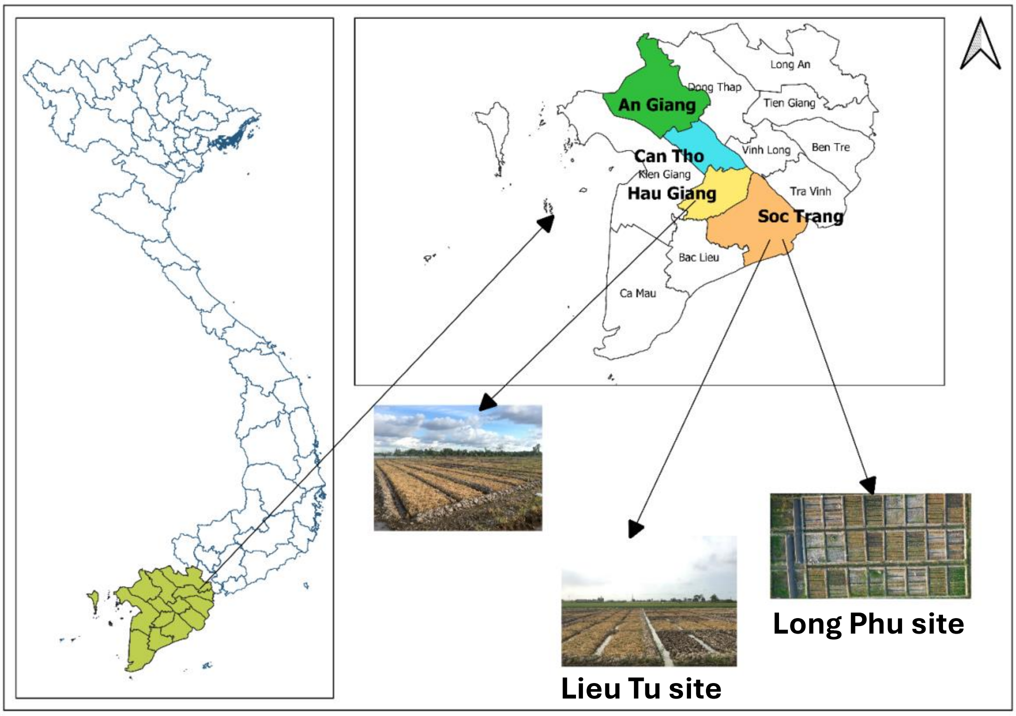

Fig. 1. The field experiment layout at Lieu Tu and Long Phu site

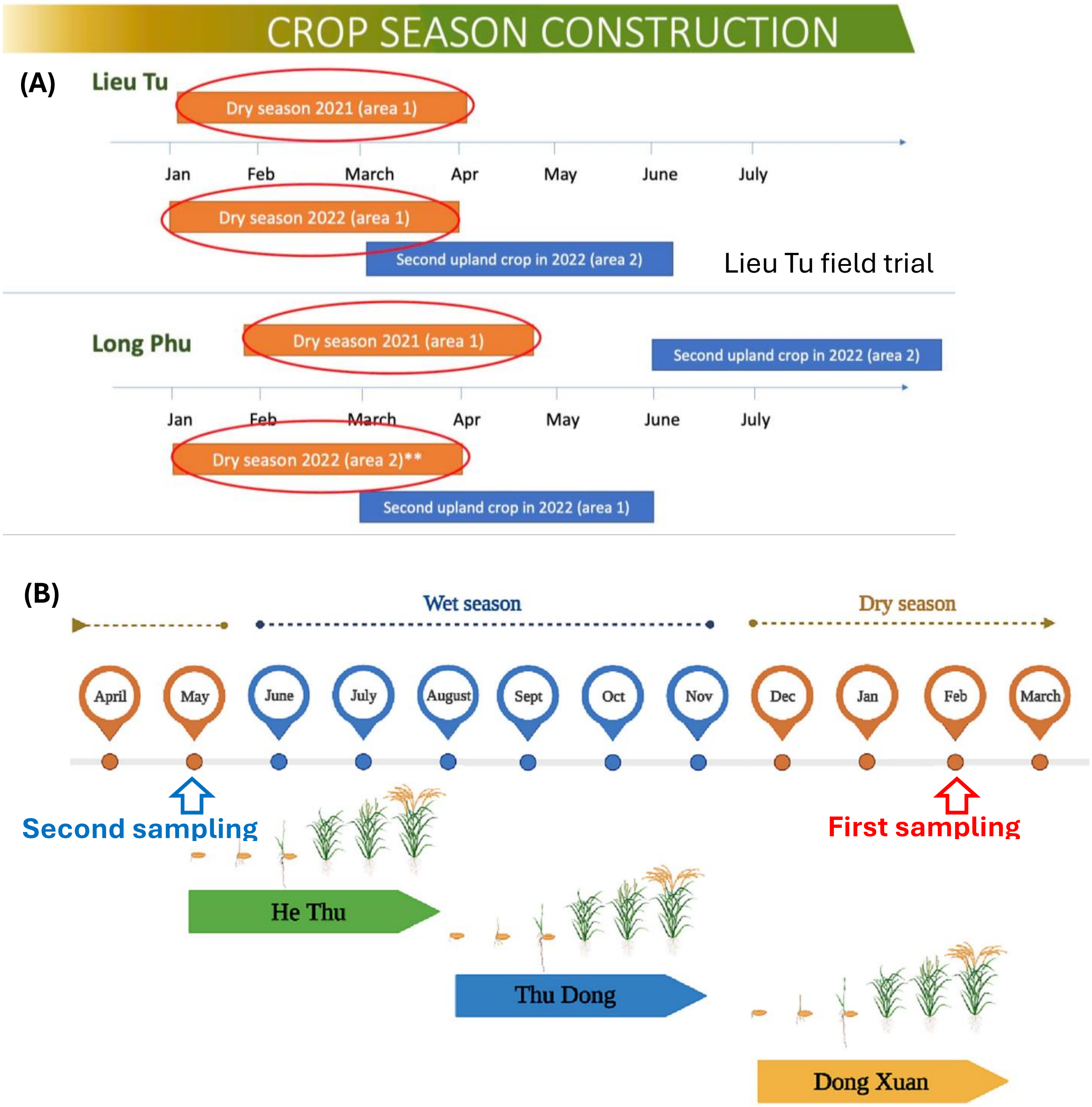

Fig. 2. Cropping seasons in triple-rice intensive systems in the VMD (A) and sampling point (B), adapted by Kaveney (2023). First sampling (February, 2023) is beginning of cowpea crop (rice was previous crop), second sampling (May, 2024) is end of cowpea crop. **Note:** in Fig. A, the orange color indicates the dry season in the VMD and the blue color indicates the duration of upland crops.

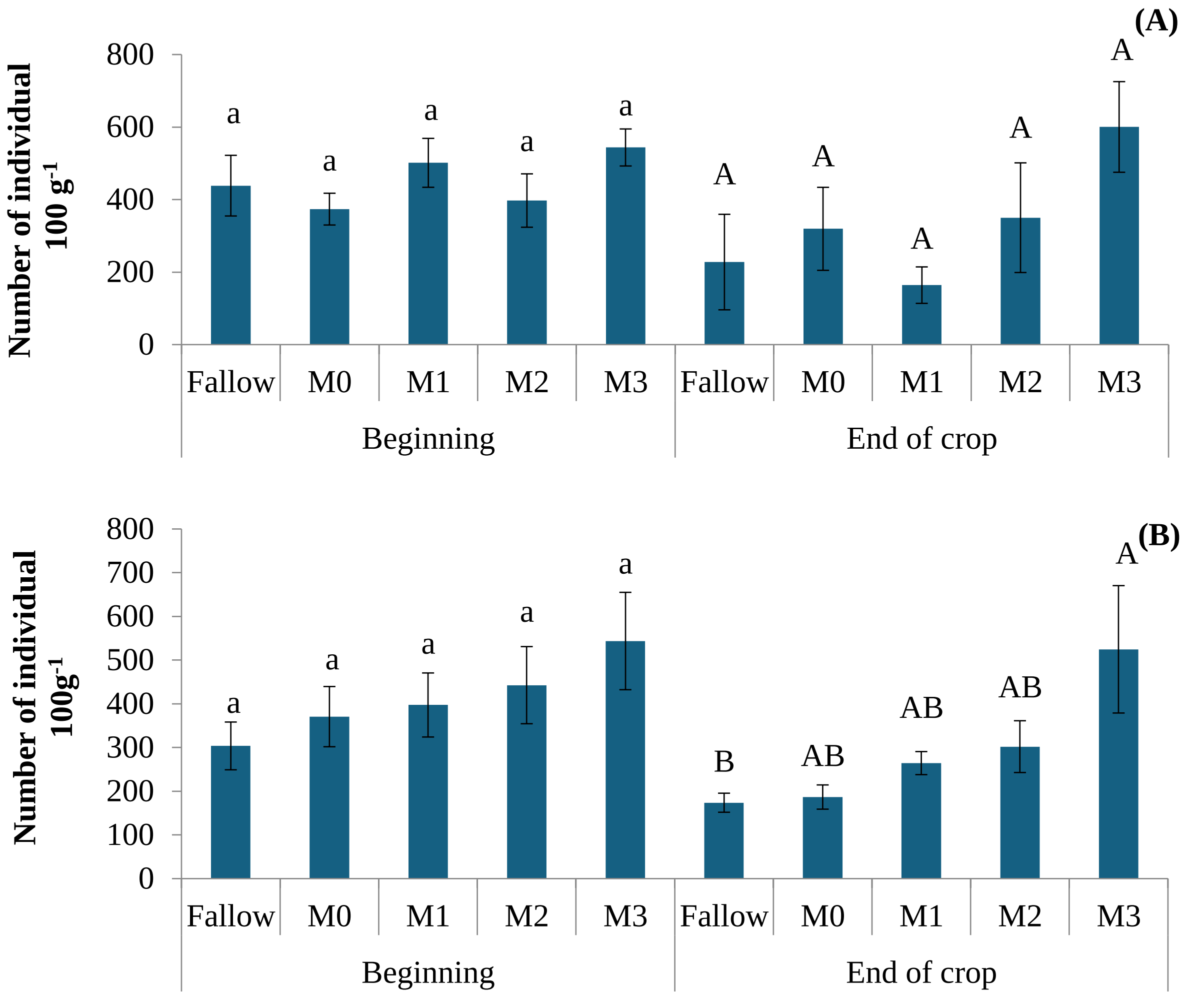

Fig. 3. Abundance (mean±SE, n=3) under rice straw mulching at Lieu Tu (A) and Long Phu (B) field trials, Soc Trang province. Different letters indicate the significant differences among treatments at $p < 0.05$ by Tukey HSD test.

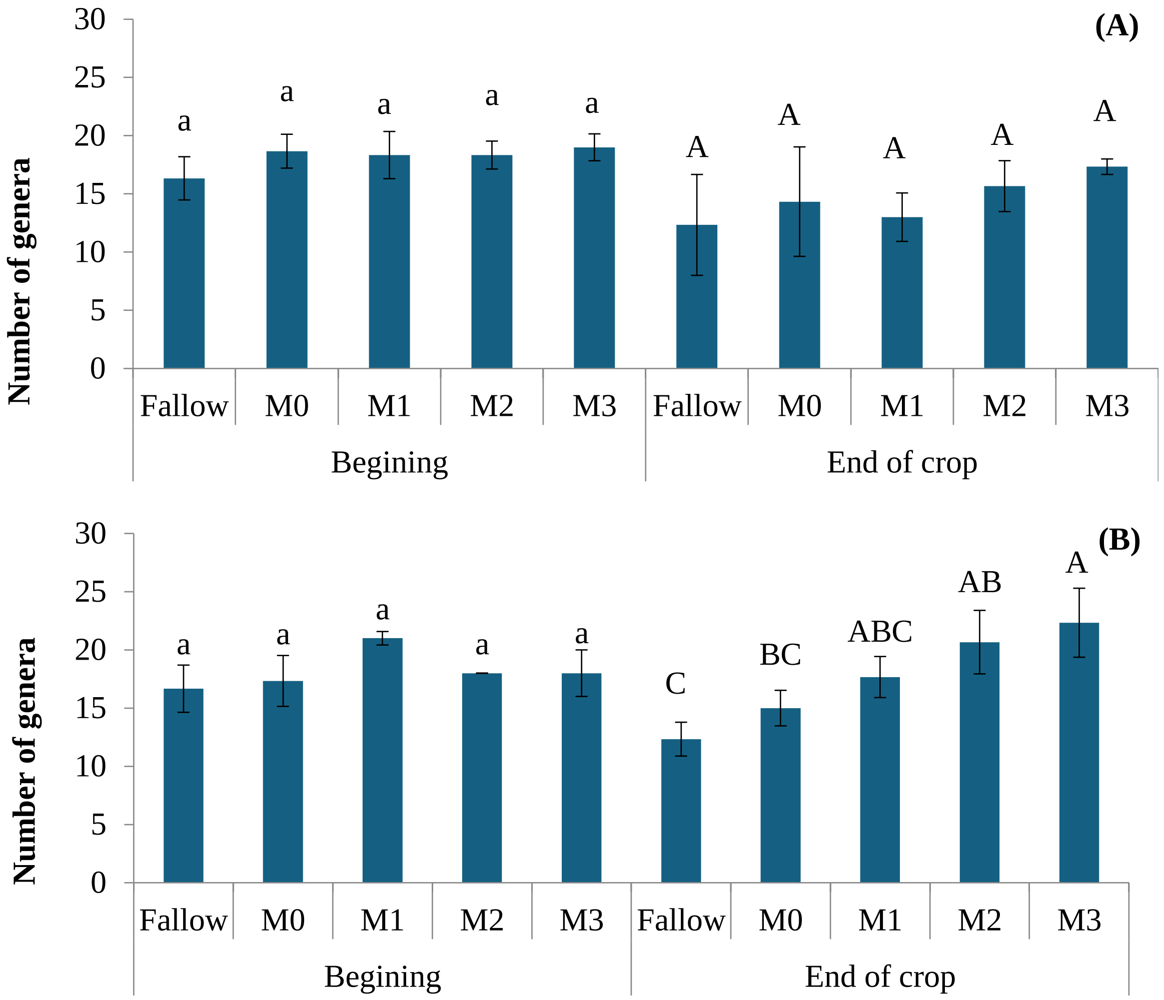

Fig. 4. Number of genera (mean±SE, n=3) under rice straw mulching at Lieu Tu (A) and Long Phu (B) field trials, Soc Trang province. Different letters indicate the significant differences among treatments at $p < 0.05$ by Tukey HSD test.

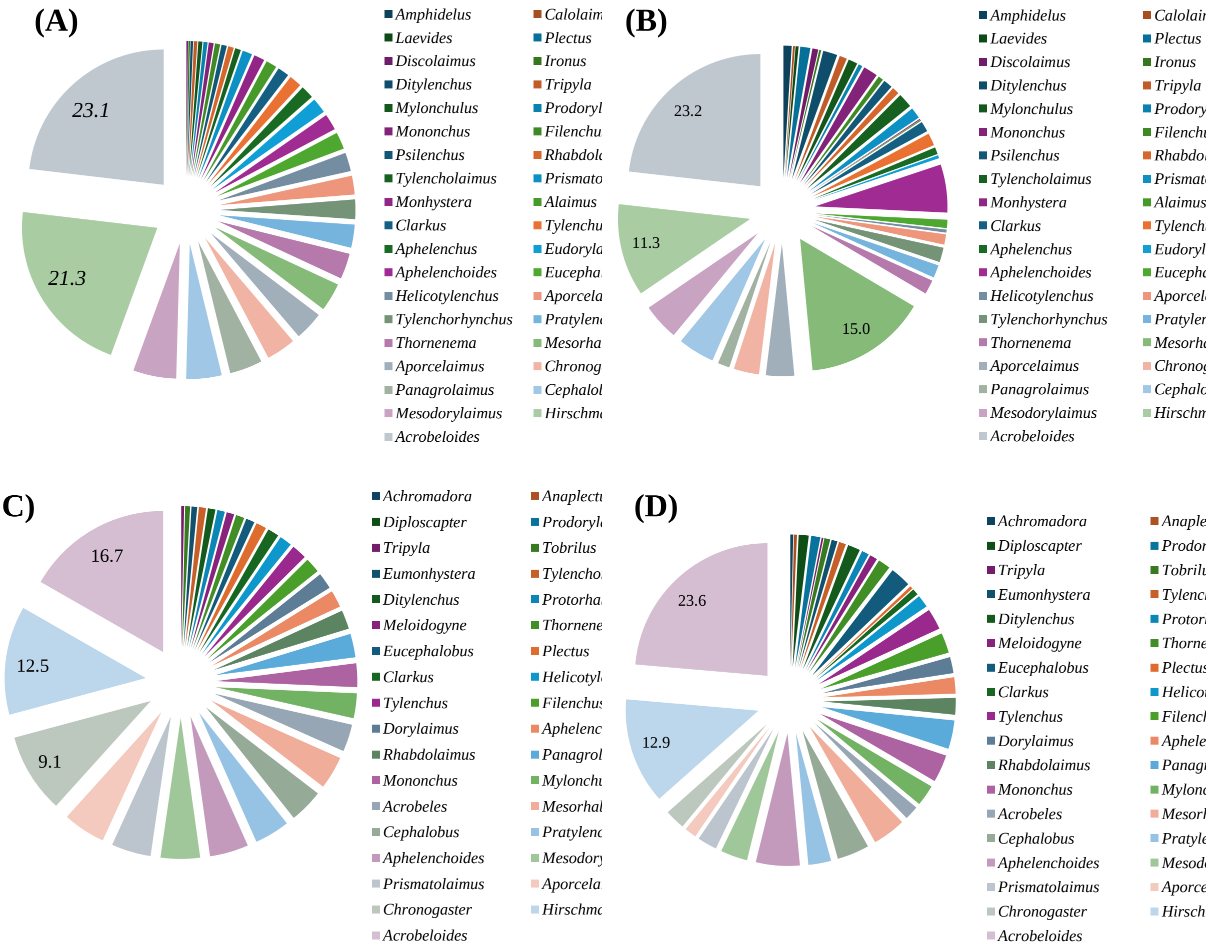

Fig. 5. Proportion (n=15) of each genera in 100g dry soil at the beginning and the end of cowpea crop in Lieu Tu (A, B) and Long Phu (C, D), respectively.

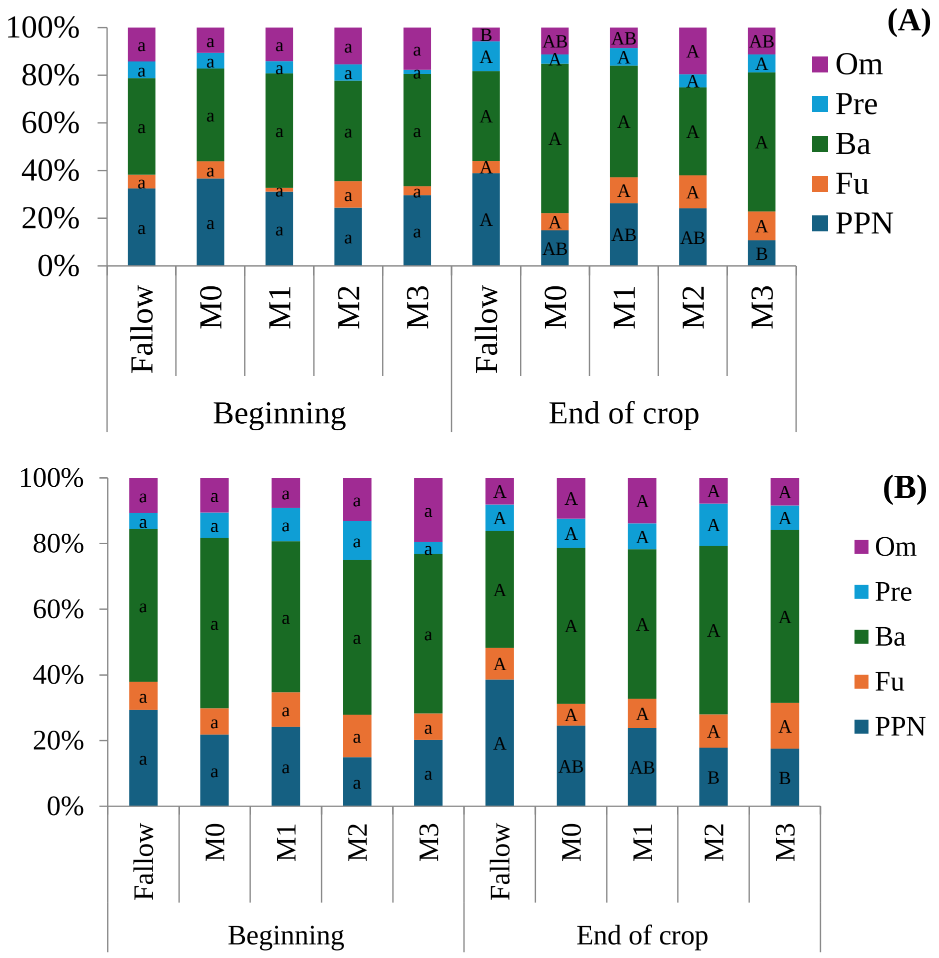

Fig. 6. Proportion of each trophic structure (mean, n = 3) in soils at the beginning and end of the cowpea cropping season in Lieu Tu (A) and Long Phu (B). Different letters indicate significant differences among treatments at $p < 0.05$, as determined by the Tukey HSD test. Trophic structures include omnivores (Om), predators (Pre), bacterivores (Ba), fungivores (Fu), and plant-parasitic nematodes (PPN).

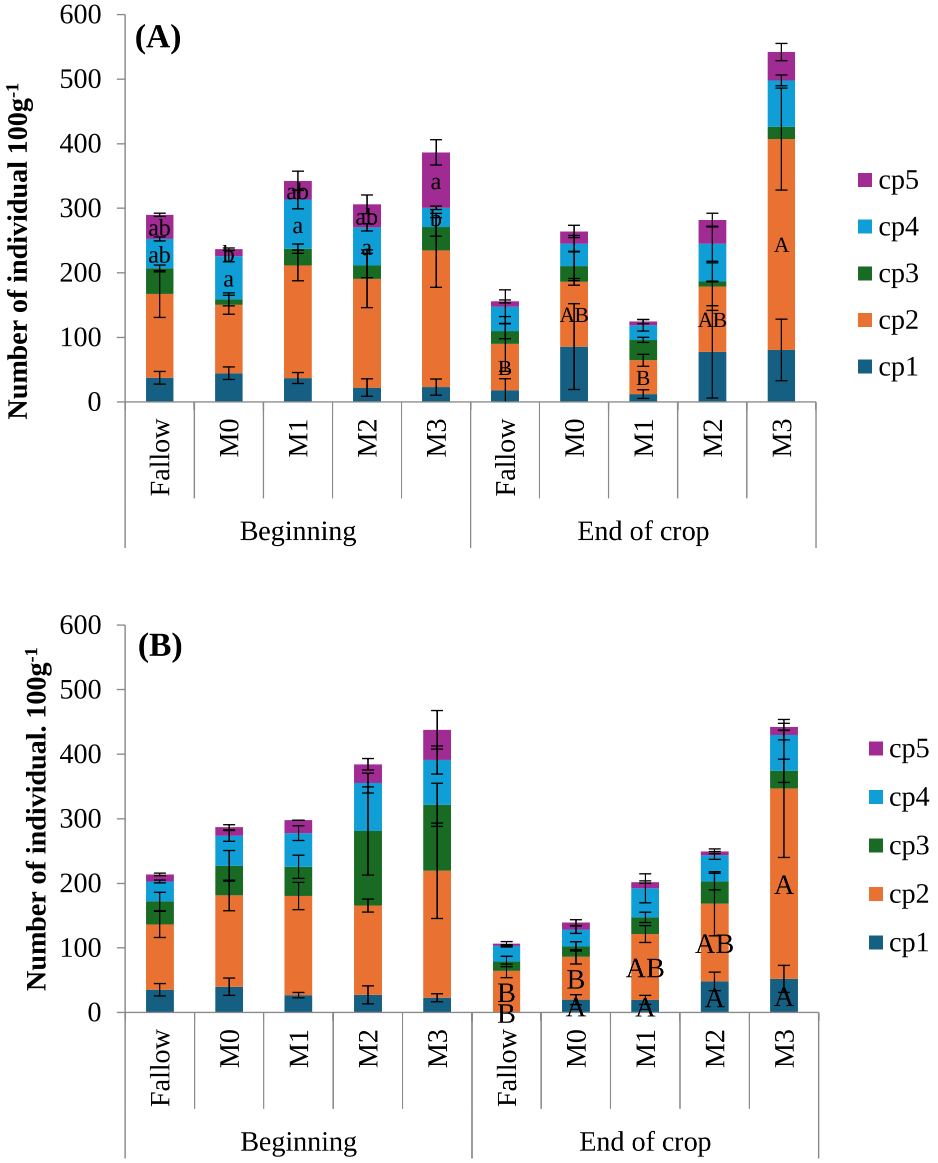

Fig. 7. Abundance of each functional guilds, cp (colonizer-persister) (mean±SE, n = 3) in soils at the beginning and end of the cowpea cropping season in Lieu Tu (A) and Long Phu (B). Different letters indicate significant differences among treatments at $p < 0.05$, as determined by the Tukey HSD test. **Notes:** nematodes of cp-1 have short generation time, high fecundity, and are mainly bacterivorous that feed on enriched media. Nematodes of cp-2, cp-3, and cp-4 have longer generation times, greater sensitivity to adverse conditions and soil disturbance, and are mainly bacterivorous, fungivorous, predator, and small omnivorous. Nematodes of cp-5 have the longest generation time, largest body, lowest fecundity, greatest sensitivity to soil disturbance, and are mainly omnivorous and predator (Ferris et al., 2001).

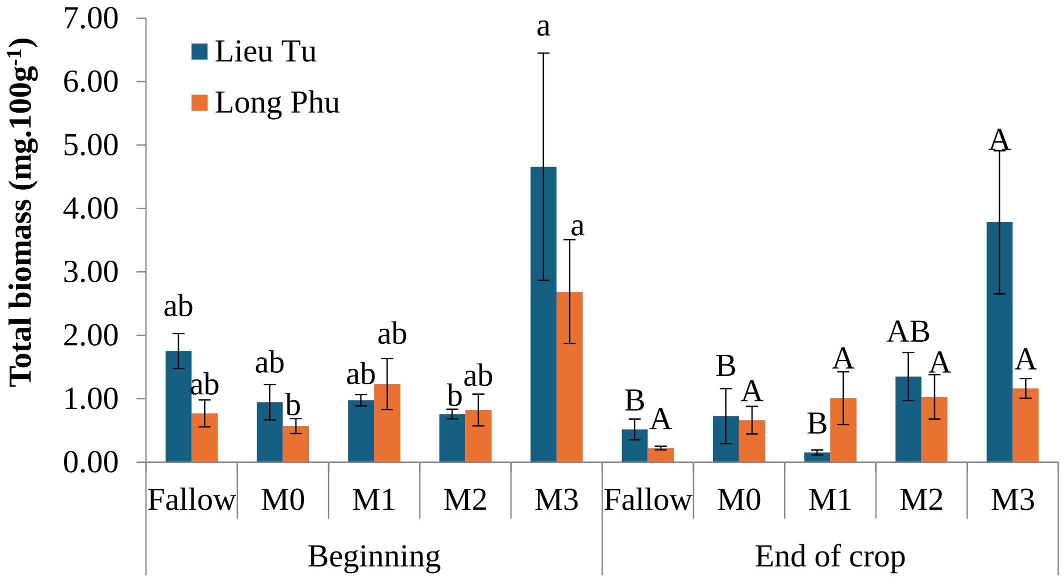

Fig. 8. The biomass of nematodes (mean±SE, n = 3) in soils from Lieu Tu (A) and Long Phu (B). Different lowercase and uppercase letters indicate significant differences among treatments at $p < 0.05$ at the beginning and end of the season, respectively, within each trial, as determined by the Tukey HSD test.

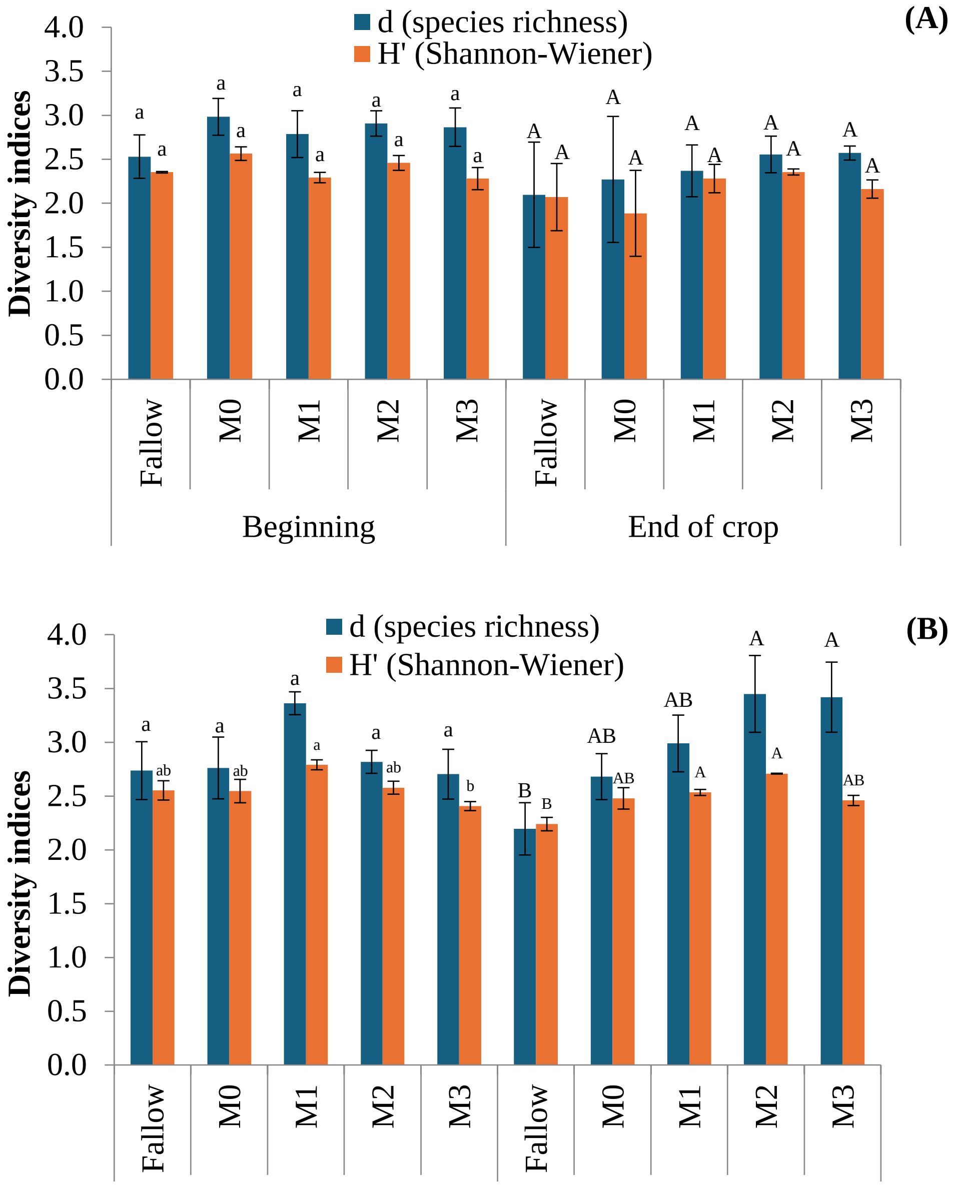

Fig. 9. Diversity indices of the nematode community (mean, n = 3) in soils from Lieu Tu (A) and Long Phu (B). Different lowercase and uppercase letters indicate significant differences among treatments at $p < 0.05$ at the beginning and end of the season, respectively, within each trial, as determined by the Tukey HSD test.

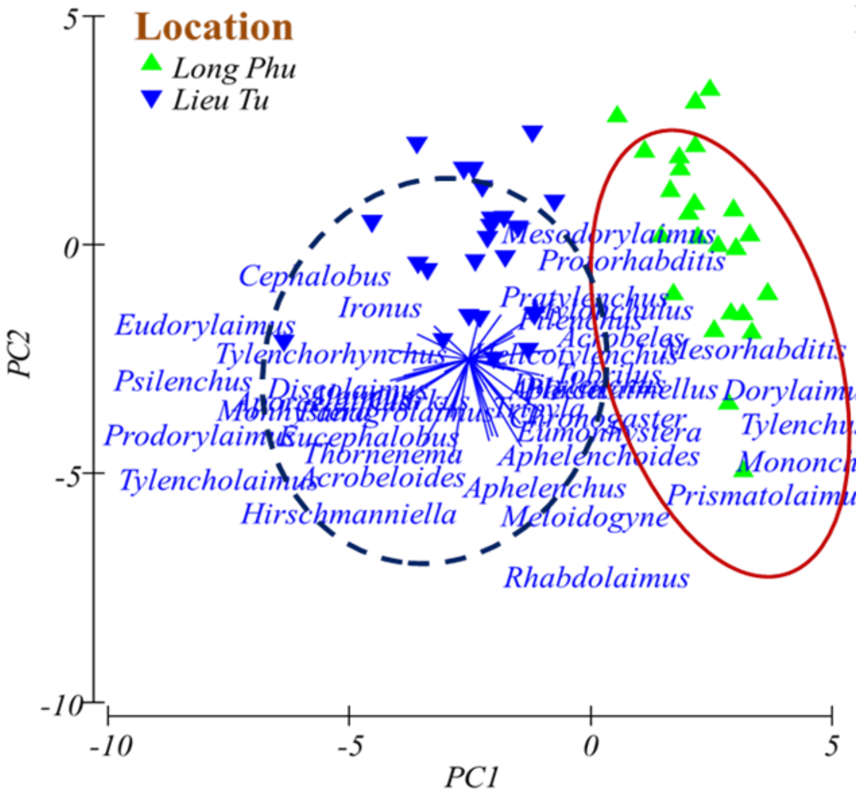

Fig. 10. Principal component analysis based on the community of nematodes in Long Phu and Lieu Tu.

Table 1. Initial soil properties at two field trials in Lieu Tu and Long Phu site

Soil properties	Lieu Tu site	Long Phu site
Sand (%)	1.50	1.60
Silt (%)	54.4	50.7
Clay (%)	44.1	47.7
Soil texture (0-15 cm)	Silty clay	Silty clay
Bulk density (g cm^{-3})	1.21	1.1
$pH_{(H2O)\ 1:5}$	5.32	5.3
$EC_{1:5}$ (mS cm^{-1})	1.22	0.5
ECe (mS cm^{-1})	7.83	3.23
Exchangeable K (cmol (+) kg^{-1})	0.619	0.664
Exchangeable Na (cmol (+) kg^{-1})	2.87	2.26
Exchangeable Ca (cmol (+) kg^{-1})	1.78	2.63
Exchangeable h Mg (cmol (+) kg^{-1})	10.1	11.5
CEC (cmol (+) kg^{-1})	15.9	17.3
ESP (%)	18.0	13.1
C total (g kg^{-1})	13.8	17.8
N total (g kg^{-1})	1.25	1.56
P total (g kg^{-1})	0.294	0.38
Available N (mg kg^{-1})	31.1	20.4
Available P (mg kg^{-1})	5.04	7.73
Available Si (mg kg^{-1})	96.5	141
	Saline–sodic soil	Slightly saline

Table 2. Metabolic footprints (mean±SE, n = 3) of nematodes community among treatments in Lieu Tu and Long Phu trial. Different letters indicate significant differences among treatments, as determined by the Tukey HSD test. ns-not significant.

The metabolic footprints (µgC.100g^{-1}) of nematodes community among treatments in Lieu Tu trial

Time	Treatment	Composite footprint	Enrichment footprint	Structure footprint	Herbivore footprint	Fungivore footprint	Bacterivore footprint	Predator footprint	Omnivore footprint
Beginning	Fallow	288.6±47.7ab	11.9±3.2a	180.7±22.1ab	60.7±16.6a	2.1±0.7a	50±11.8a	23.4±6.5a	152.4±27.4ab
	M0	180.8±33b	12.8±2.9a	95.4±32.4b	47.9±6.4a	2.4±0.4a	38.6±7.6a	17.8±5.7a	74.1±30.5b
	M1	223.6±11.7b	10.1±2.6a	101.4±24.3b	59.1±24.7a	0.9±0.7a	67±10.2a	20.5±5.5a	76±31.1b
	M2	173.2±24.2b	8.5±2a	84±2.3b	32.9±4.1a	3.8±2.4a	56.1±22.1a	20.8±4.6a	59.6±5.1b
	M3	623.4±194.2a	6.7±3.2a	493.4±199.9a	56.5±4.2a	1.9±0.4a	77.7±23.2a	7.9±1.6a	479.4±201.2a
	p-value	**0.008**	**ns**	**0.019**	**ns**	**ns**	**ns**	**ns**	**0.028**
End of crop	Fallow	108.1±40b	6.4±6.1a	58.7±19.3ab	27.7±9.7	3.1±2.8	23.2±13.8ab	22.7±7.6	31.4±17.2ab
	M0	144.8±65.9ab	22.4±16.9a	75.8±49ab	20.4±8.8	2.1±1.1	50±10.2ab	13.8±9.1	58.5±39.4ab
	M1	44.2±11.1b	4.4±1.6a	19.2±7.7b	12±2.5	1.6±0.2	16.4±4.3b	8.7±4.8	5.5±1.8b
	M2	220.6±21.1ab	21±18a	155±39ab	21.1±2	4.2±0.9	42.6±24.1ab	21±8.2	131.8±47ab
	M3	542.8±131.3a	25.1±13.8a	402±121.9a	22.8±4.1	7.6±3.4	116.4±32.4a	36.8±5.4	359.3±125.2a
	p-value	**0.007**	**ns**	**0.025**	**ns**	**ns**	**0.038**	**ns**	**0.030**

The metabolic footprints (µgC.100g^{-1}) of nematodes community among treatments in Long Phu trial

Time	Trearment	Composite footprint	Enrichment footprint	Structure footprint	Herbivore footprint	Fungivore footprint	Bacterivore footprint	Predator footprint	Omnivore footprint
Beginning	Fallow	152.4±22.4a	10.1±1.2a	89.1±25.7a	30.9±3.6a	2.4±0.3a	34.5±5.6a	13.1±5.5a	71.5±31.6a
	M0	138.5±26.9a	12.2±4.1a	67±14.5a	26.9±10.1a	2.5±1a	46.2±10.2a	26.8±15.5a	36±5.2a
	M1	222.7±64.2a	11±3.1a	79.6±17.7a	101.9±50.9a	4.7±2.1a	40.5±5.7a	35.7±17.5a	39.9±15.5a
	M2	182.1±47a	9.4±1.6a	123.7±37.8a	19.2±3.1a	3.2±1.5a	50.1±18a	35.9±13a	73.6±28.2a
	M3	440.9±122.7a	8.6±2a	225.2±88.5a	153.9±64.2a	2.9±1.4a	71.7±31.2a	15.5±3.1a	197±86.4a
	p-value	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
End of crop	Fallow	59.5±6.6b	1±0.2b	22.3±4.5a	24.4±4.7a	1±0.2a	13.2±2.5b	11.1±3.8a	9.8±2.5a
	M0	118.9±29.2ab	5.1±2.2b	56.9±19.8a	40.7±28.9a	1.4±0.7a	21.5±2.6ab	11.9±1.5a	43.4±20.2a
	M1	176.1±60.3ab	6.5±1.8ab	77.3±31.6a	65.5±26.5a	1.7±0.2a	33.9±4.6ab	17.1±8.6a	57.9±26.6a
	M2	179.6±51.7ab	13.4±3.5a	82.4±24.9a	58.9±18.2a	2.7±1.3a	38.1±17.1ab	22.3±8.2a	57.6±32.3a
	M3	242.2±34.9a	19.1±9.3a	99.8±27.2a	48.9±19.8a	7.3±4a	89.2±34.1a	28.1±5.7a	68.6±28.2a
	p-value	**0.05**	**0.016**	**ns**	**ns**	**ns**	**0.019**	**ns**	**ns**