
Is halophyte species growing in the vicinity of the shrimp ponds a promising agri-aquaculture system for shrimp ponds remediation in New Caledonia?

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Abstract :

Plant culture integration within aquaculture activities is a topic of recent interest with economic and environmental benefits. Shrimp farming activities generate nutrient-rich waste trapped in the sediments of farming ponds or release in the mangrove area. Thus, we investigate if the halophytes species naturally growing around the pond can use nitrogen and carbon from shrimp farming for remediation purposes. Halophyte biomasses and sediments influenced by shrimp farm effluents, were collected in two farms in New-Caledonia. All samples were analyzed for their C and N stable isotopic composition and N content. Higher $\delta^{15}\text{N}$ values were found in plants influenced by shrimp farm water thus evidenced their abilities to take nutrient derived from shrimp farming. Deep root species *Chenopodium murale*, *Atriplex jubata*, *Suaeda australis* and *Enchylaena tomentosa* appears more efficient for shrimp pond remediation. This work demonstrates that halophytes cultivation in shrimp ponds with sediments, could be effective for the pond's remediation.

Highlights

► $\delta^{15}\text{N}$ values of halophyte and sediment are influenced by shrimp farm waters. ► Halophytes can take advantage of shrimp farm waste and feed pellet for their growth. ► Halophyte culture in shrimp pond could be a promising solution for pond remediation.

Keywords : Nutrient recycling, Aquaculture waste, Isotope ecology, Halophytes, Shrimp farming

1. Introduction

Pond bottom conditions are particularly critical for shrimp species which live at the sediment-water interface (Avnimelech & Ritvo, 2003). Intensive and semi-intensive shrimp farming requires a daily food supply, however only a minor part of the nutrients is assimilated in shrimp biomass (Funge-Smith & Briggs, 1998; Joesting et al., 2016; Dien et al., 2019; Ni et al., 2020). In fact, Funge-Smith and Briggs (1998) estimated that in intensive farming, shrimp assimilate only 23.1% of nitrogen (N) and 11.8% of phosphorus (P) from the feed pellet. In the case of semi-intensive farming, Páez-Osuna et al., (1997) estimated that 46,7% of N and 7.4% of P from the feed pellet was assimilated by shrimps. Consequently, during the rearing, a large proportion of uneaten feed becomes sedimented in the pond bottom. In addition to the uneaten feed, feces and dead plankton also accumulate in sediment (Páez-Osuna et al., 1997; Funge-Smith & Briggs, 1998; Hargreaves, 1998; Boyd & Clay, 1998). Then, the excessive organic matter accumulation in pond bottom may result in anaerobic conditions and a concomitant release of toxic metabolites into the overlying water (Hargreaves, 1998; Hargrave, Holmer & Newcombe, 2008). Poor ponds bottom conditions and water quality affect well-being of the reared shrimps and may increase diseases prevalence and hence resulting in mass mortality and unsustainable production (Kautsky et al., 2000).

Usually, at the end of each cycle of production, drainage and drying of pond are done to prepare next round of aquaculture production. This process aims to discard polluted water and allows the aeration of the pond bottom soil to enhance microbial decomposition of the accumulated organic matter (Yang et al., 2017). However, pond drying is not always beneficial and consumes time that could be used in producing shrimp (Boyd & Pippopinyo, 1994). In addition, the discharge of shrimp farm effluent

could have deleterious effects on the receiving ecosystem and is usually directly released in aquatic ecosystem without treatments (Cardoso-Mohedano et al., 2016). Depending on the farm intensification degree (stocking density) and on the management (feed pellet composition, fertilizers uses or not), pond effluents could contain elevated nutrient levels, suspended solids, organic matter and exhibit a high biochemical oxygen demand (Boyd & Tucker, 1998; Pàez-Osuna, 2001). This could modify the trophic state of the receiving ecosystem by causing eutrophication, oxygen depletion or also changes in benthic macrofauna (Pàez-Osuna, 2001).

In New-Caledonia, semi-intensive shrimp ponds are built on saltpans upstream mangrove forest (Della Patrona & Brun, 2009) and pond effluents are discharges into the mangrove prior their release into adjacent waters (Fig 1). Previous studies showed, especially through meiofauna distribution and assemblage, that shrimp farms effluent has low impact on the environmental quality of adjacent mangrove of New Caledonia (Molnar et al., 2013; Debenay et al., 2015; Della Patrona et al., 2016) and the natural biofilter capacity of mangrove is considering limiting effluent impacts on the surrounding lagoon and coral reef (Molnar, 2012).

The observations made on 17 active shrimps' farms in New Caledonia showed that most of all high parts of the internal earth dikes are colonized by perennials and/or short living species of halophytic plants (Della Patrona et al., 2016). Halophytes are extremely salt tolerant plants that play an important role in protecting ecosystems due to their remediation abilities (Etesami & Beattie, 2018). Moreover, they are valuable products for different fields (food, fuel, fiber, cosmetics) (Koyrobr & Lieth, 2011; Ventura et al., 2011; Glenn et al., 2013; Norman, Masters & Barrett-Lennard, 2013; Hasanuzzaman et al., 2014; Sharma et al., 2016; Certain et al., 2021). Due to their

salinity tolerance, halophytes seem to be strong candidates to be associated with shrimp farming in brackish and marine waters for either pond bioremediation and/or food uses (Fierro-Sañudo, Rodríguez-Montes de Oca & Páez-Osuna, 2020). Indeed, since the last decades, integrated agriculture-aquaculture farming system emerge as a more sustainable system production (Prein, 2002; FAO, 2019). This technique is based on the use of the waste from one system as inputs to another's (Prein, 2002). Aquaculture wastes typically contain ammonia, nitrate, and phosphorus which are essential for plant growth. Then, they could be a viable nutrient source for plants production (Joesting et al., 2016). In this way, nutrients trapped in (i) the organic-rich mud, (ii) the sediment or (iii) present in the water could be reused as fertilizers for crop production (Ahmed, Thompson & Glaser, 2019; Islam et al., 2020).

Integrated shrimp-plant culture has been a topic of recent interest. Fierro-Sañudo et al. (2020), found that shrimp-vegetable rotational farming system with vegetables in tidal ponds during the idle period significantly reduce nitrogen and total phosphorus in soil and water. Shrimp-vegetable rotational farming system not only remediates the shrimp farming ecosystem but also increases profits by planting vegetables (Ni et al., 2020). The economic and ecological importance of halophytes species must be considered for the urgent improvement of sediments and effluents quality of shrimp ponds. First, it appears necessary to determine if halophytes species can take advantage of the carbon and nitrogen sources from shrimp farming activities.

One of the methods used to investigate the identification of organic matter from anthropogenic sources, such as municipal sludges, agriculture and/or aquaculture impacts is the use of stable isotope analysis which becoming widespread in many disciplines. Indeed, various sources of nitrogen have a distinguishable $\delta^{15}\text{N}$ signature that could be integrated into the component of the food web. In Piñón-Gimate et al.,

(2009), they investigated the $\delta^{15}\text{N}$ signature of macroalgae species from coastal lagoons influenced by N sources derived from various anthropogenic activities (sewage, shrimp farming, agriculture) and supporting algal growths. Depending on the most nitrogen source contributor in the sampling location, they evidenced significant variations of $\delta^{15}\text{N}$ macroalgae values. Indeed, $\delta^{15}\text{N}$ values reflect the N source used by plant (Högberg, 1997). For instance, in red alga (*Catenella nipae*), Costanzo, O'Donohue & Dennison (2004) have shown that the $\delta^{15}\text{N}$ values was close-range to $\delta^{15}\text{N}$ signatures of shrimp farm effluents. In Lin & Fong (2008), high $\delta^{15}\text{N}$ values of macroalgae (*Acanthophora spicifera*) were found near the shrimp farm and lead them to conclude that shrimp farm effluents are the dominant nutrient source for the alga. However, to date no data are available on halophyte species growing around shrimp farms. In addition, the $\delta^{15}\text{N}$ signature might also be affected by the plant physiology as $^{15}\text{N}/^{14}\text{N}$ fractionation could occur during uptake, assimilation, and transport of nitrogen (Högberg, 1997; Evans, 2001). Thus, $\delta^{15}\text{N}$ is also a physiological proxy likewise $\delta^{13}\text{C}$ that integrating plant photosynthetic performance (Dawson et al., 2002).

Taking together the specificities of the New-Caledonian shrimp farming management and the flora distribution around the shrimp farm area, it is important to understand how halophytes species can naturally grow in these zones. Thus, as our study was conducted in New Caledonia, we decided to principally focus on ecological solution mean for organically enriched pond bottoms remediation rather than shrimps farm effluent pollution (Funge-Smith & Briggs, 1998; Ni et al., 2020).

The aim of the study was to determine whether shrimp farm effluent influenced the stable carbon and nitrogen isotopic composition and N contents of six halophytes species naturally growing under influence of New Caledonia shrimp ponds. The study was conduct in a purpose of farther use of halophytes species for shrimp pond

bioremediation. In addition, this work aims to provide information about halophyte physiology since nitrogen and carbon isotopic composition is related to plant metabolism.

2. Materials and Methods

2.1. *Shrimp farm management*

This study takes place in two semi-intensive shrimp farms (18-20 post larvae/m²) separated by 16 km away from each other on the West coast of New Caledonia. They are both using seawater collected in the same bay and both are constructed behind mangrove area. Farm A has total surface of 18 ha and starts operation in 1983 (38 years old at the writing time) and Farm B is 30 ha and starts in 2005 (16 years old at the writing time). The investigate shrimp farms produce the blue shrimp *Litopenaeus stylirostris* with an average yield of shrimps between 1.5 to 2.5 tons per hectare.

For both farms, ponds are filled by seawater and water renewal is ensured by a water-exchange system that supply seawater from the lagoon. Usually, the lagoon seawater has a salinity of 35 - 38 ‰ (except after heavy rainfall) and an average temperature range between 20°C to 27°C (Della Patrona & Brun, 2009). Shrimp farm activity generally starts in beginning of hot season (September to January) for 6-8 months after which the farm proceeds a 3-month break (June–August) that allows the drainage and drying of ponds (Della Patrona & Brun, 2009). Water renewal and/or aeration systems such as paddlewheel systems allow to maintain water column oxygenation. The Aquaculture Stewardship Council (ASC, <https://www.asc-aqua.org/>), an international label created in 2010 by the WWF, encourages responsible farming practices and canvasses farmers to follow their ASC shrimp standard. For example, they require that the farmer ensure to maintain oxygen levels above 3 ppm, which was done in the 2 studied farms.

Farm A water renewal is of $18\% \pm 3\%$ with 134 m^3 water/kg shrimp produced whereas Farm B water renewal is $12\% \pm 2\%$ with 108 m^3 water/kg shrimp produced. In Farm B, additional oxygenation is provided by aerators.

During the active period of the shrimp farm, daily effluents water discharges reaching up to 30% of pond volumes (Della Patrona & Brun, 2009) are released in little sandy-made channels at multiple points around farm area and then in the mangrove and saltmarsh (Fig.1). At the end of the rearing, Farm B ponds are drained and dry whereas in Farm A, farmer lets the tides sweeping in and out the pond to rinse it before drying.

Shrimps are daily feed (1 to 4 times per day) throughout the rearing period with inputs increasing over the rearing cycle as the shrimp grow. Feeding rate is controlled by means of several feeders placed on the pond bottom which are checked one hour after being place. Both farms use for several years the same pellet brand, and the pellet composition has not been changed (according to the pellet factory) thereby facilitate the interpretation of feed pellet isotopic signature without ambiguity.

2.2 Site description

The objectives are to see (i) if halophytes species that naturally grow at vicinity of shrimp farm can use shrimp pond nutrients,(ii) if there are differences between Farm A and Farm B and (iii) if they the shrimp pond effluents have an impact on their nitrogen isotopic signature. In these aims, we sampled halophytes in areas with different exposition degrees to pond nutrients: inside shrimp pond, outside shrimp pond and in a saltmarsh around the two studied shrimp farms (Fig.1).

Inside Farm A and Farm B shrimp pond, halophytes are supposed to be the most exposed to pond nutrient as they grow inside the slope dike of earthen ponds and are

continuously splashed and/or submerged by nutrient rich seawater and waves from the ponds during the 7 to 8 months active rearing period (Fig.1).

Outside shrimp pond, halophytes are exposed to farm effluents (Fig.1) and nutrient concentration supposed to be more diluted than inside shrimp pond. Indeed, outside Farm A, halophytes are continuously splashed and/or submerged by a mixture of shrimp farm effluents (during farm activities) and by seawater (during mean and high tides). Outside Farm B, halophytes are influenced by effluents of four shrimp ponds and rarely influenced by tides.

In the sampled saltmarsh, halophytes growth is rule out any possible influence of shrimp farm effluents, anthropogenic or livestock impact. The saltmarsh represents our control condition and was selected by its similarity with saltpans used for the construction of both shrimp ponds. In this control area, halophytes are frequently submerged by mean and high tides.

2.3. Sample collection:

Six halophyte species were identified at the vicinity of shrimp farms and in the control saltmarsh, and are: *Sarcocornia quinqueflora*, *Suaeda australis*, *Sesuvium portulacastrum*, *Atriplex jubata*, *Enchylaena tomentosa*, and *Chenopodium murale*.

We have collected both the aboveground and belowground biomass when it was possible for each species depending to their natural occurrence on the sites, to evaluate if each part (e.g.: aboveground and belowground) of the plant had the same C and N isotopic signature. Collected biomasses will allow us to see if these six halophytes' species are able to use shrimp pond nutrients for their growth and if there is storage preference between the aboveground or belowground part of the plant; and

regarding the sampling site (e.g.: control saltmarsh, inside the pond and outside the pond).

From field observations, *Suaeda australis*, *Atriplex jubata*, *Enchylaena tomentosa* and *Chenopodium murale* have a deep root system whereas *Sesuvium portulacastrum* and *Sarcocornia quinqueflora* have a shallower root system.

Each replicate consists of a pool of at least five healthy individual plants (young seedlings and senescent plants were discarded). Samples were stored in sealed plastic bags and refrigerated immediately after collection until further processing. In the laboratory, all samples were washed with deionized water to remove salts and sediment particles. Aboveground and belowground biomass of halophytes was separated and stored at -20°C. In total, 42 aboveground and 36 belowground halophytes biomasses were harvested.

Simultaneously to plants, sediment samples (0–20 cm) from the rooting depth were also collected to investigate if the sediment could inform about the nutrient availability for the plants as they used their roots system to incorporate their nutrient from their surrounding sediments. One replicate consists in a composite sample of 5 to 10 sediment samples, depending on the surface area of sampling site. Triplicate were made for each sample site. At the time of sampling, soils were in moist condition. In total, for the 5-sampling area, we collected 15 sediment samples.

Sample of fresh shrimp feed pellet has been collected from the farmers. Farm A and B use the same feed pellet for ten years. The pellets contain 35 % protein, 8 % lipids, 3 % cellulose, 7 % ash, 10 % moisture (composition gave by SICA Manufacturer, New Caledonia).

2.3. Samples preparation and analyses

Samples were freeze-dried for at least 72 h. Dried samples were ground and homogenized in a fine powder. For analysis, 3.00 to 3.10 mg of aboveground or belowground halophyte biomass powdered samples were packed into tin capsules, 1 mg of powder for feed pellet and 5 mg for sediment. All samples were analysed in triplicate.

The analyses were performed by the Laboratory of Analytical Means (LAMA/ISO 9001, Nouméa, New Caledonia) using an elemental analyzer-IRMS (Integra2 Sercon isotope ratio mass spectrometer) to determine $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, total carbon and total nitrogen in all samples. The analytical precisions of the analyser were checked using the IAEA-600 caffeine standard (IAEA Nucleus) and were 0.13‰ for ^{15}N , and 0.45 ‰ for ^{13}C . Isotope composition are expressed in δ notation relative to standard reference materials (atmospheric N_2 for $\delta^{15}\text{N}$ and Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$).

2.4. Statistical analyses

All statistical analyses were performed with R software. Significance levels were set at $p < 0.05$. First, a paired sample *t*-test was performed to compare average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in aboveground and belowground halophyte biomass. A non-parametric test of Kruskal-Wallis followed by a pairwise Wilcoxon rank sum tests was used to show mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ differences for each species and sediment between sampled areas and feed pellet. This test was also performed to show significant variations of total nitrogen and C/N in halophyte biomasses following sampled areas. A correlation analysis of Kendall was performed to evaluate relation between $\delta^{15}\text{N}$ and nitrogen content of halophyte biomass. In order to see if the sample area impacts the above- or belowground isotopic signature of the halophytes, two Principal Component Analysis

(PCA) were performed. Mann-Whitney test was done to evaluate if the N levels in the plants was dependent to the sample sites.

3. Results

3.1 $\delta^{13}\text{C}$ isotopic signature

3.1.1 $\delta^{13}\text{C}$ isotopic signature between aboveground and belowground biomass

In aboveground biomass, a $\delta^{13}\text{C}$ depletion compared to belowground is observed in *Suaeda australis* and *Enchylaena tomentosa* collected in their control areas; on contrary $\delta^{13}\text{C}$ is slightly enriched in *Sarcocornia quinqueflora* aboveground biomass collected in control saltmarsh (Tab.1) In the control zone where *Sesuvium portulacastrum* grows, mean $\delta^{13}\text{C}$ value is almost the same between the two plant parts as the $\delta^{13}\text{C}$ variation between the above- and the belowground is 0.01 ‰ (Tab.1). For all species, $\delta^{13}\text{C}$ variations observed between the two plant-parts are significantly different through the sampled area (Tab.1). For instance, aboveground biomass of *Suaeda australis* is $\delta^{13}\text{C}$ depleted compared to belowground in all sampled area and this depletion is significantly higher in control area. Whereas *Sesuvium portulacastrum* aboveground biomass is significantly $\delta^{13}\text{C}$ enriched inside Farm B area (Tab.1). However, all species combined, the $\delta^{13}\text{C}$ variation observed between the two plant-parts seems not clearly influenced by the sampled area (Fig.SI 1). Indeed, in the PCA (Fig.2) the $\delta^{13}\text{C}$ of the belowground and $\delta^{13}\text{C}$ of the aboveground biomass are not correlated to the sampled area.

Broadly, for all species combined, means $\delta^{13}\text{C}$ values are not significantly different (p-value > 0.05) between aboveground (-24.89 ‰ \pm 3.77) and belowground (-23.95 ‰ \pm

3.69) biomass (Fig.SI 2A). Consequently, for the rest of the study, we will consider the $\delta^{13}\text{C}$ of the whole halophyte biomass.

3.1.2 $\delta^{13}\text{C}$ isotopic signature in feed pellet, sediment and halophyte following sampled site.

The $\delta^{13}\text{C}$ values of the studied halophytes varies between -24 to -28 ‰ except for the *Atriplex jubata* exhibiting -13 ‰ signature (Fig.3).

Halophyte $\delta^{13}\text{C}$ value varies in small proportion between sampled sites (Fig.2 and Fig. 3). Highest variations are in *Sarcocornia quinqueflora* and *Sesuvium portulacastrum* with respectively 1.9 and 1.5‰ variation between control and the outside area of the farm A. Overall, among all the halophytes collected, the variations between sites are generally less than 1 ‰.

Suaeda australis and *Chenopodium murale* $\delta^{13}\text{C}$ are not significantly different between the sampled sites (Fig.3). *Sesuvium portulacastrum* $\delta^{13}\text{C}$ is higher in control but does not significantly vary between farm areas. All species have a $\delta^{13}\text{C}$ isotopic signature significantly higher than feed pellet (Fig.3).

For sediments, $\delta^{13}\text{C}$ values in farm are significantly (p -value <0.05) more negative than the control (Fig.3) and lower than the feed pellet (-23.08 ± 0.06 ‰). In farms, it ranges from -12.84 to -18.01 ‰ and the most negative values are in sediments of the inside sediment of the farm B (-18.01 ± 0.26 ‰). The $\delta^{13}\text{C}$ variations between sampled areas are more pronounced than in halophytes' aboveground biomass.

As halophytes and sediments $\delta^{13}\text{C}$ values are significantly different than feed pellet and varies little in halophyte biomass, it seems not useful to track the organic matter origin in this study.

3.2. $\delta^{15}\text{N}$ isotopic signature

3.2.1. $\delta^{15}\text{N}$ isotopic signature between aboveground and belowground biomass

As shown in the Table 1, aboveground $\delta^{15}\text{N}$ values of halophytes are at least 0.38‰ higher than belowground biomass thus showing a $\delta^{15}\text{N}$ enrichment in aboveground except in the case of *Sesuvium portulacastrum* outside farm B. Highest $\delta^{15}\text{N}$ variations are in *Enchylaena tomentosa* control and *Atriplex jubata* inside farm B with aboveground 4.32 and 5.48 ‰ enrich than belowground respectively (Tab.1).

In some species, $\delta^{15}\text{N}$ differences between the two plant-parts may also varies significantly following the sampled area (Tab.1). Thus, $\delta^{15}\text{N}$ enrichment of *Sesuvium portulacastrum* aboveground biomass is significantly higher inside farm B whereas *Sarcocornia quinqueflora* $\delta^{15}\text{N}$ aboveground enrichment is significantly higher in control area. $\delta^{15}\text{N}$ variation in *Suaeda australis* is not significantly different between sampled areas. However, all species combined, $\delta^{15}\text{N}$ variation along the 2 parts is not clearly influenced by the areas as display in the figure (Fig.SI 1).

All species combined, mean $\delta^{15}\text{N}$ is significantly different between the two plant-parts (Fig.SI 2B). Aboveground biomasses show the highest $\delta^{15}\text{N}$ and seem then to be the best proxy to reflect shrimp farm influence. In addition, PCA shows that $\delta^{15}\text{N}$ aboveground biomass of halophyte is clearly more influenced by the areas than belowground (Fig. 2). Therefore, we choose to principally to focus on the aboveground $\delta^{15}\text{N}$ signature of the halophytes for the rest of the study.

3.2.2 $\delta^{15}\text{N}$ isotopic signature in feed pellet, sediment, and halophyte according to the sampled site.

The mean $\delta^{15}\text{N}$ of *Sarcocornia quinqueflora*, *Sesuvium portulacastrum*, *Suaeda australis* is significantly higher in farm areas than in control (Fig.4). For those species, higher difference of $\delta^{15}\text{N}$ compares to control are in *Suaeda australis* growing in the inside pond area of the farm A (4.4‰), *Sarcocornia quinqueflora* outside farm A (3.28‰) and *Sesuvium portulacastrum* inside farm B (6.9‰).

For a given species, $\delta^{15}\text{N}$ values can vary significantly between sampled farm areas (Fig.4). For instance, *Suaeda australis* inside and outside Farm A exhibits $\delta^{15}\text{N}$ values at 6.08 ± 1.21 ‰ and 4.81 ± 0.21 ‰ respectively. As well, *Sesuvium portulacastrum* inside and outside farm B $\delta^{15}\text{N}$ values are respectively 7.12 ± 0.93 ‰ and 5.00 ± 0.60 ‰.

Mean $\delta^{15}\text{N}$ also vary between species collected at the same area. Indeed, $\delta^{15}\text{N}$ values of *Chenopodium murale*, *Suaeda australis*, and *Sesuvium portulacastrum* from inside farm A are respectively 8.25 ± 1.75 ‰, 6.08 ± 1.12 ‰ and 4.34 ± 1.38 ‰. Highest mean $\delta^{15}\text{N}$ values are found in *Chenopodium murale* inside farm B (9.21 ± 0.96 ‰).

In the control area, halophyte mean $\delta^{15}\text{N}$ are significantly lower than feed pellet (Fig.4). However, in farms areas, *Sesuvium portulacastrum* (inside farm A, outside farm B), *Suaeda australis* (inside farm A, outside farm B) and *Sarcocornia quinqueflora* (outside farm A) have mean $\delta^{15}\text{N}$ values not significantly different from feed pellet.

In sediment, means $\delta^{15}\text{N}$ from farm B are significantly higher than control but not significantly different from feed pellet.

Moreover, the results highlight a positive moderate but significant correlation ($R = 0.37$) between the $\delta^{15}\text{N}$ values of the halophytes biomass and the nitrogen content (N) (Fig.5).

3.3. Total nitrogen and C/N ratios in halophytes and sediment following sampled site.

In control area, significant highest nitrogen levels are found in *Enchylaena tomentosa* and *Suaeda australis* compared to *Sesuvium portulacastrum* and *Sarcocornia quinqueflora* (Tab.2). Conversely, lowest C/N ratios are found in *Enchylaena tomentosa* and *Suaeda australis* compared to *Sesuvium portulacastrum* and *Sarcocornia quinqueflora*. In shrimp farm areas, significant highest levels of nitrogen are found in *Suaeda australis*, *Atriplex jubata* and *Chenopodium murale* compared to *Sesuvium portulacastrum* and *Sarcocornia quinqueflora* (Tab.2). Outside farm A area, *Sarcocornia quinqueflora* has significant lower nitrogen level and higher C/N ratios than *Sesuvium portulacastrum*. Highest level of nitrogen and lower C/N ratio are found in *Chenopodium murale* inside farm B.

All species combined, nitrogen levels found in control area are significantly lower than in areas influenced by shrimp farming, according to the Mann-Whitney test ($p\text{-value} < 0.005$) (Tab.SI 1).

The nitrogen levels of *Sesuvium portulacastrum* and *Suaeda australis* increase in shrimp farm areas compared to control area (Tab.2). An opposite trend is observed for the C/N ratio. Nitrogen level of *Sesuvium portulacastrum* and *Chenopodium murale* is significantly higher in farm B than farm A.

For both farm, nitrogen level found inside the farm area sediment are significantly higher than outside farm sediment (Tab.SI 2). Nitrogen level from control saltmarsh

sediment is not significantly different than in shrimp farm sediment. However, C/N ratio of the control saltmarsh sediment is significantly higher than inside and outside farm A and B area. C/N ratio were significantly lower inside farm B area (Tab. SI 2).

3.4. $\delta^{15}\text{N}$ and nitrogen content in Farms A and B

Sesuvium portulacastrum is the only species found everywhere, it is then use for halophytes comparisons between farms. The *Sesuvium portulacastrum* $\delta^{15}\text{N}$ values are significantly higher in farm B than in farm A (Tab.3). Highest sediment $\delta^{15}\text{N}$ values were also found in farm B. Highest $\delta^{15}\text{N}$ aboveground biomass values are observed for inside farm B, while weakest values were observed for outside farm A. This is also the case for sediment values which are significantly higher in inside farm B than outside farm A (Tab.3). No statistically difference of mean $\delta^{15}\text{N}$ aboveground biomass values were observed between inside farm A and outside farm B (Tab.3).

Nitrogen contents of aboveground biomass farm B are significantly higher than in farm A (Tab.2). The highest values are found in outside farm B (23.00 ± 0.12 mg/g) and the lowest in outside farm A (12.23 mg/g) (Tab.2).

4. Discussion

4.1. Isotopic signature, C/N ratios and organic matter source

4.1.1. In the Sediment

In a recent study Martínez-Durazo et al. (2019) showed that sediments from lagoon receiving both anthropogenic and shrimp farms effluents have a $\delta^{13}\text{C}$ signature more depleted (-21.91‰) than sediment from unimpacted lagoon (-17.21‰). The same trends were observed in (Grego, Malej & Troch, 2020), with a sediment $\delta^{13}\text{C}$ signature more depleted under fish farms compared to reference site (-24.4‰ versus -21.8‰). It appears that terrestrial vegetable in fish feed pellet composition led to depleted $\delta^{13}\text{C}$ values (Yokoyama, Abo & Ishihi, 2006). Moreover, due to their metabolic processes, fish faeces are more $\delta^{13}\text{C}$ depleted than fish feed (Mazzola & Sarà, 2001). Thus generally, fish cages wastes are characterised by depleted $\delta^{13}\text{C}$ values (Holmer et al., 2007). Although our work focuses on saltmarsh-based shrimp farms we also identified more depleted $\delta^{13}\text{C}$ values in shrimp pond sediments compared to the control saltmarsh sediments. This observation highlights potential influences of feed pellet in shrimp pond sediment despite their difference in $\delta^{13}\text{C}$ values. The C/N ratio is generally considered as indicator to distinguish the origins of organic matter in sediment. Indeed, algae (phytoplankton and zooplankton) are rich in protein and typically have a C/N ratio of 4-10; while vascular land plant mainly composed of cellulose and lignin have a C/N ratio of 15 or more (Thornton & McManus, 1994; Rumolo et al., 2011; Mahapatra, Chanakya & Ramachandra, 2011). Thus, sediment collected inside and outside shrimp pond, with a C/N ratio range between 8 and 13 evidence a dominant influence of marine organic matter inputs, probably derived from shrimp farming. Whereas in the saltmarsh sediment considered as our control, the C/N ratio is close to vascular plant and evidenced organic matter inputs dominated by terrestrial origin. This observation

is also supported by the statistical test showing the saltmarsh control is significantly different from the other area (Tab.SI 1).

Nitrogen isotopic signature also underlined feed pellet influences on pond sediment. In natural ecosystems, high amount of available nitrogen increases isotopic $\delta^{15}\text{N}$ discrimination (Craine et al., 2015). Nitrification, denitrification, and ammonification processes directly influence the quantity of available nitrogen and lead to a sediment enriched in ^{15}N . Microorganism's process, leaching, ammonia volatilization also increase the ^{15}N pool of soil (Hobbie & Ouimette, 2009; Gritcan et al., 2016; Tanu et al., 2020).

Our results show that $\delta^{15}\text{N}$ concentrations in Farm B sediment is higher than in control saltmarsh. Thus, it appears, in agreement with Molnar et al. (2013) that in our study $\delta^{15}\text{N}$ is imported from the pond ecosystem. Indeed, feed pellet representing the major shrimp pond nitrogen source (76% in semi-intensive and 78% in intensive farming) but it was estimated that only 46.7% and 23.1% of feed pellet nitrogen, in semi-intensive and intensive farming respectively, would be harvest by shrimp (Páez-Osuna et al., 1997; Funge-Smith & Briggs, 1998). Then, considerable levels of pond nitrogen could be released through effluent (estimated at 36.7% in semi-intensive farming) and potentially accumulate in ponds sediment (estimated at 24% in intensive farming) (Páez-Osuna et al., 1997; Funge-Smith & Briggs, 1998). In addition, $\delta^{15}\text{N}$ values of farm B sediment was not statistically different from feed pellet confirming it traces on sediment. Contrary to what we might be expected, nitrogen levels found in the sediment inside farm B were not significantly different from saltmarsh and were even lower outside farm B. The same trend was observed in the outside farm A sediment. We suppose that during tide and effluents release, a frequent and easier nitrogen leaching occurs due to the water circulation, that could explain the lowest levels of

nitrogen found outside the farm areas. Moreover, the use of nitrogen by microorganisms, volatilization and denitrification process could also significantly lower the sediment N values (Mahapatra, Chanakya & Ramachandra, 2011). Thus, all those factors make difficult to evidenced nitrogen accumulation in sediment.

As different combinations of nitrogen sources may produce similar $\delta^{15}\text{N}$ values, additional information on factors affecting local nitrogen dynamics could be required to assert that nitrogen isotopic signature is solely influenced by shrimp farming and feed pellet (Viana & Bode, 2013; Wong et al., 2022). Indeed, anthropogenic factors (agricultural, sewage, urbanization) could be a source of nitrogen input in coastal waters (Wong et al., 2022). New Caledonia highland has low anthropogenic pressure on environment as urbanized area and population densities are small with an average of 15 people per km^2 . However, there is insufficient wastewater treatment and network that led to marine pollution particularly in the proximity of the main city Noumea (Payri et al., 2019). As our study is far away from main city Noumea, we could suppose that the shrimp pond seawater pumped into lagoon is not influenced as could be the seawater in the vicinity of Noumea. Nevertheless, it might be a little influenced by anthropogenic activities such as crop farming or sewage from the nearby city. However, more studies are needed to evaluate the role of the river discharged and lagoon current on the water composition of the bay. Apart anthropogenic factor, coastal seawaters nitrogen inputs could also be related to upwelling natural event. In New-Caledonia, nutrient upwelling event has been observed however nutrient enrichment was low and principally confined in the open ocean outside the lagoon system (Ganachaud et al., 2010; Fuchs et al., 2013).

4.1.2. In halophyte biomasses

In a previous study, Cullain et al. (2018) found less negative $\delta^{13}\text{C}$ farther away from finfish farm in eelgrass (*Zostera marina*). Similar patterns of less negative $\delta^{13}\text{C}$ values farther away from the source have been observed in other seagrass species beds which receive organic material from fish farms (Vizzini & Mazzola, 2004; Holmer et al., 2007). Unlikely, but in line with several studies, we found very little variation in $\delta^{13}\text{C}$ isotopic signature of halophytes (mostly less than 1 ‰) along our sampled sites differently influenced by shrimp farm effluent (Jones et al., 2001; Vizzini & Mazzola, 2006; Serrano-Grijalva et al., 2011). Moreover, $\delta^{13}\text{C}$ of aquatic plants like seagrass are influenced by the uses of dissolved inorganic carbon whereas this is not the case for terrestrial plants (Finlay, 2008; Szpak et al., 2013). Those observations might indicate that in our study, $\delta^{13}\text{C}$ doesn't allow us to determine origin of organic matter in the halophyte's biomasses.

In our study, all halophyte specimens collected from different shrimp farm areas had higher $\delta^{15}\text{N}$ isotopic composition than those sampled in the control saltmarsh. Our results confirmed those of Lin & Fong (2008), who found higher $\delta^{15}\text{N}$ values in the tissues of red macroalga *Acanthophora spicifera* exposed to Polynesian shrimp farm effluents. As well, Pérez et al., (2020) found higher $\delta^{15}\text{N}$ values in roots and shoots of mangroves trees nearby a Peruvian shrimp farm area. The $\delta^{15}\text{N}$ increasing in halophyte biomass would be linked to assimilation of higher $\delta^{15}\text{N}$ concentration in shrimp farm sediment than control saltmarsh as precedingly explained (4.1.1). Using a large data set, we have demonstrated a positive correlation between $\delta^{15}\text{N}$ and nitrogen content of halophyte tissues confirming the pattern of increasing $\delta^{15}\text{N}$ values following nitrogen inputs in environment. These results agreed with those of studies carried out on other salt tolerant species such as *Posidonia oceanica* (Pérez et al.,

2008), *Spartina alterniflora* (Kinney & Valiela, 2018), Hawaiian coastal wetland species (Bruland & MacKenzie, 2010) and Okinawan mangrove tree species (Tanu et al., 2020). Nitrogen levels found in halophyte biomasses of *Sesuvium portulacastrum* and *Suaeda australis* were higher in shrimp farm area than in the control saltmarsh supporting the assumption of higher nitrogen inputs in farm areas. In addition, the decreasing of halophyte biomasses C/N ratios in farm areas also evidence higher nitrogen availability as this ratio responds to environmental conditions and plants generally exhibit low C/N ratios when nutrients are less limited (Zhang et al., 2020). In addition, some halophyte species such as *Suaeda australis*, *Sesuvium portulacastrum*, *Sarcocornia quinqueflora*, growing under the influence of shrimp farm effluent, have a $\delta^{15}\text{N}$ value not significantly different from the feed pellet suggesting that these halophyte species used the feed pellets as the main nutrient source if there is no external nitrogen source influencing isotopic signature (4.1.1).

4.1.3. Isotopic signature related to plant physiology

Although $\delta^{13}\text{C}$ didn't allow us to trace organic matter source in halophyte, it is well known that $\delta^{13}\text{C}$ may provide useful information about plant photosynthetic metabolism (Barbour & Hanson, 2009; Tcherkez, Mahé & Hodges, 2011). According to Dawson et al. (2002), C3 plants have $\delta^{13}\text{C}$ values comprising between -20‰ and -35‰ and C4 plants between -11‰ and -15‰. Thus, in our study, *Chenopodium murale* (-28‰), *Enchylaena tomentosa* (-25‰), *Sarcocornia quinqueflora* (-25‰), *Sesuvium portulacastrum* (-24‰) and *Suaeda australis* (-27‰) show a C3 metabolism to fix carbon whereas *Atriplex jubata* (-13‰) uses a C4 metabolism. The lower $\delta^{13}\text{C}$ values in C3 plants mean that they discriminate more strongly against heavier isotopes (^{13}C) than C4 plants during carbon fixation. The plant carbon isotopic composition may also

be influenced by many environmental factors such as water stress, nutrient availability, irradiance, soil temperature and salinity (Chaudhary, Kim & Kang, 2018). All these factors are characterised by high temporal variability and may affect plant leaf stomatal aperture controlling discrimination against ^{13}C during photosynthesis (Szpak et al., 2013). Thus, halophytes $\delta^{13}\text{C}$ can be influenced by many other key parameters than organic matter sources making difficult its interpretation.

In our study, aboveground biomasses of the investigated halophytes had significant higher values of $\delta^{15}\text{N}$ than belowground biomasses. According to Evans (2001), $\delta^{15}\text{N}$ variations in plant are strongly influenced by nitrogen sources and assimilation patterns. The transport of enriched ^{15}N inorganic nitrogen from root to shoot could explain the $\delta^{15}\text{N}$ variation observed between the two-plant parts (Kalcsits, Buschhaus & Guy, 2014). Indeed, after a first assimilation of inorganic nitrogen by the roots, the remaining pool of nitrogen available for shoot assimilation is enriched in $\delta^{15}\text{N}$ as the result of the higher affinity of the roots enzymatic complex for the ^{14}N . Then, according to Kalcsits, Buschhaus & Guy (2014), an increasing $\delta^{15}\text{N}$ difference between shoot and root indicates a greater proportion of assimilation occurring in the leaves. However, the $\delta^{15}\text{N}$ plant discrimination is controlled by relationship between nitrogen availability and plant demand and occur when plant nitrogen demand is relatively low compared to nitrogen availability (Evans, 2001; Kalcsits, Buschhaus & Guy, 2014).

Several studies found that $\delta^{15}\text{N}$ difference between the two-plant parts could also depend on the assimilated nitrogen form (NO_3^- or NH_4^+) (Evans et al., 1996; Evans, 2001) and the nitrogen form preferences would be associated to the prevailing N-form in the natural habitat of the plant (Brix, Dyhr-Jensen & Lorenzen, 2002). However, in natural ecosystem, plant can uptake both two nitrogen forms, which generally makes

difficult the explanations, and the interpretations of plant $\delta^{15}\text{N}$ intra-variation solely based on nitrogen forms.

4.3. *Farming practices influences on $\delta^{15}\text{N}$ signature*

As the two studied shrimp farms are in the same bay and separated by only 16 km, they are under the same climatic influences, allowing $\delta^{15}\text{N}$ comparisons. In our study, significantly higher $\delta^{15}\text{N}$ values of *Sesuvium portulacastrum* were reported in farm B pond compared to farm A pond and outlet area. We would have expected to find higher $\delta^{15}\text{N}$ isotopic signatures in halophytes biomasses of farm A because, as the oldest, it theoretically accumulates more nitrogen in its sediment than farm B. Even if the farmers use same shrimp density (18-20 post larvae/m²) and the same feed pellet, we assume that this seemingly contradictory finding is likely due to the different management practices that farmers have followed for years and still follow (i.e., pond water renewal, pond dryness, aerators use). Generally, 5% to 25% of the pond water is renewed per day throughout rearing (Della Patrona & Brun, 2009). From the data provided by farmers, we firstly highlighted that farm A water renewals (average 18% \pm 3% water volume/day and 134 m³ water /kg shrimp produced) were significantly higher than in farm B (average 12 \pm 2 %water volume /day and 108 m³ water/kg shrimp). However, the farm B used aerators to compensate lower oxygen levels. Moreover, the two farms adopt different “drying” inter crop pond strategies. During the inter-crop period (4-5 months), farmer A lets the infrequently highest tides sweeping in and out to rinse the pond then let it dry, while farmer B seals out the inlet/outlet system to avoid tides to enter and so than the ponds can dry out. Those practices suggest that nutrient wastes are more dilute in farm A and could explain the lower $\delta^{15}\text{N}$ concentration

observed. Thus, $\delta^{15}\text{N}$ provides a useful tool capable to point out management differences between the two farms through plant isotopic signature.

4.4. Selection of appropriate halophytes species

The shape and extent of plant root system plays essential role in the efficiency of nutrient and water acquisition from soil. Bouma et al., (2001), have worked on root system topology from seven halophytes species depending their elevations on the saltmarsh. They evidenced that species from high marsh part like genus *Atriplex* has a dichotomous branching root conferring greater capacity to acquire nutrient while species from low marsh part like *Salicornia europaea* has short lateral roots that may aid survival in anoxic sediment by aerating the root under inundated conditions.

Although they concluded that root typology is not necessarily an adaptative trait of saltmarsh plants, we found higher nitrogen levels in *Suaeda australis*, *Atriplex jubata*, *Enchyalena tomentosa* and *Chenopodium murale* which are mainly found in high part of New Caledonia saltmarsh and have a deeper root system (personal observation, Pic.SI 1). Whereas lowest nitrogen levels were found in the biomass of two mat-forming grow species *Sarcocornia quinqueflora* and *Sesuvium portulacastrum* with a shallower root system (personal observation, Pic.SI 1), frequently submerged by tide and, in the case of *Sesuvium portulacastrum*, present in lower part of the saltmarsh (Certain, 2021). Thus, we could suppose that *Suaeda australis*, *Atriplex jubata*, *Enchylaena tomentosa*, and *Chenopodium murale* root system may have more abilities or predisposition to take up nutrients within soils horizons and being more efficient for shrimp pond bioremediation. However, further study is needed to determine i) the nitrogen capture efficiency of the studied halophyte species, ii) how much each of these halophytes could assimilate from shrimp ponds, iii) what is the most efficient species

and iv) in what proportion should the halophyte be integrated to shrimp culture for an efficient bioremediation. Indeed, previous studies determine proportion of tomato plant and lettuce that could be integrated to shrimp farming to produce without use fertilizers and to reduce nutrient losses (nitrogen and phosphorus) from shrimp monoculture. However, those studies were conducted in zero water exchange shrimp tank with low salinity which is different from our studied coastal shrimp earthen ponds with marine water (Mariscal-Lagarda et al., 2012; Mariscal-Lagarda & Páez-Osuna, 2014; Fierro-Sañudo et al., 2015). In addition, it's also important to consider the life cycle and the salinity tolerance of these halophytes for their integration to shrimp farming. In high saline environments, the use of an appropriate N-form nutrition (nitrate or ammonium) is generally beneficial for the halophyte growth and may mitigate the harmful effect of the salt exposure (Certain et al., 2021). Certain, (2021) has explored in experimental greenhouse the salinity tolerance of New Caledonia halophytes species for biosaline agriculture purpose. Thus, it was evidenced that *Sarcocornia quinqueflora* growth is unimpacted by salinity as high as seawater, whereas at high salinity *Atriplex jubata* growth is better under nitrate nutrition. For *Suaeda australis* and *Enchylaena tomentosa*, a mix of two-nitrogen form was recommended for a better growth under high salinity. This thesis work constitutes a first base for selection of halophytes integration to shrimp farming in New Caledonia.

Choices of proper halophyte species may also rely on it potential as valuable crops products that could provide an additional income for shrimp farmer. Indeed, physiological adaptation of halophytes to saline stress induce the production of different biomolecules like antioxidants, antimicrobials, anti-inflammatory, fatty acid or also carbohydrates with a great agri-food, pharmaceutical and cosmetic value (Vineeth et al., 2021). Among our studied species, potential valorisation of genus species

Sesuvium portulacastrum, *Suaeda australis*, *Sarcocornia quinqueflora* and *Atriplex* genus has been fully documented. For instance, *Sarcocornia quinqueflora* is a valuable crops as human food while *Atriplex jubata* is rather used as fodder for livestock.

In our study, the deep root species *Suaeda australis* accumulate more nitrogen in it tissues when naturally growing inside the dike of shrimp ponds. Thus, it appears efficient for shrimp pond bioremediation contrary to the shallower root species *Sesuvium portulacastrum* and *Sarcocornia quinqueflora* which have accumulated more nitrogen outside shrimp farm and saltmarsh area respectively. The other deep root species *Atriplex jubata*, *Enchylaena tomentosa* and *Chenopodium murale* also appear interesting for bioremediation because they exhibit high nitrogen levels in their tissues. Nevertheless, we lack data to compare their nitrogen accumulation between at least inside and outside the shrimp pond.

5. Conclusion

The aim of the study was to determine how shrimp farm water influence the nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotopic signature of halophyte species that naturally grow at the vicinity of shrimp ponds. We evidenced that shrimp farm water exposition has significantly increase the $\delta^{15}\text{N}$ of halophyte whereas $\delta^{13}\text{C}$ variation was principally related to plant photosynthetic metabolism. Plant $\delta^{15}\text{N}$ variation was linked to higher assimilation of nitrogen from shrimp farm water as supported through positive correlation between $\delta^{15}\text{N}$ and nitrogen levels in halophytes biomass. *Suaeda australis*, *Sesuvium portulacastrum* and *Sarcocornia quinqueflora* $\delta^{15}\text{N}$ isotopic signature was not significantly different from the feed pellet, the major source of nitrogen in the pond water. That highlight that the halophyte assimilate nutrient derived from shrimp farm activities, if there is no external nitrogen source influencing isotopic signature.

Thus, our result support the potential use of halophyte species as a solution for the bioremediation of nutrient rich shrimp pond in New-Caledonia. This study constitutes a preliminary exploration but further researches are needed to evaluate the bioremediation capacity of shrimp sediment by halophyte and first step will be to select the proper's halophyte species. From our data, deep root species such as *Chenopodium murale*, *Atriplex jubata*, *Suaeda australis* and *Enchylaena tomentosa* appear more efficient for shrimp pond nitrogen remediation. Although it needs to be experimentally tested, we hypothesis that root system penetration affects assimilation capacity as higher nitrogen levels were found in biomass of deep root species. To end, the selection of high valuable potential halophyte species integration to shrimp farming could provide an additional income to farmer.

Author's contributions:

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Nolwenn Callac: Writing – Review & Editing

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2. Materials and Methods

2.1 Site description

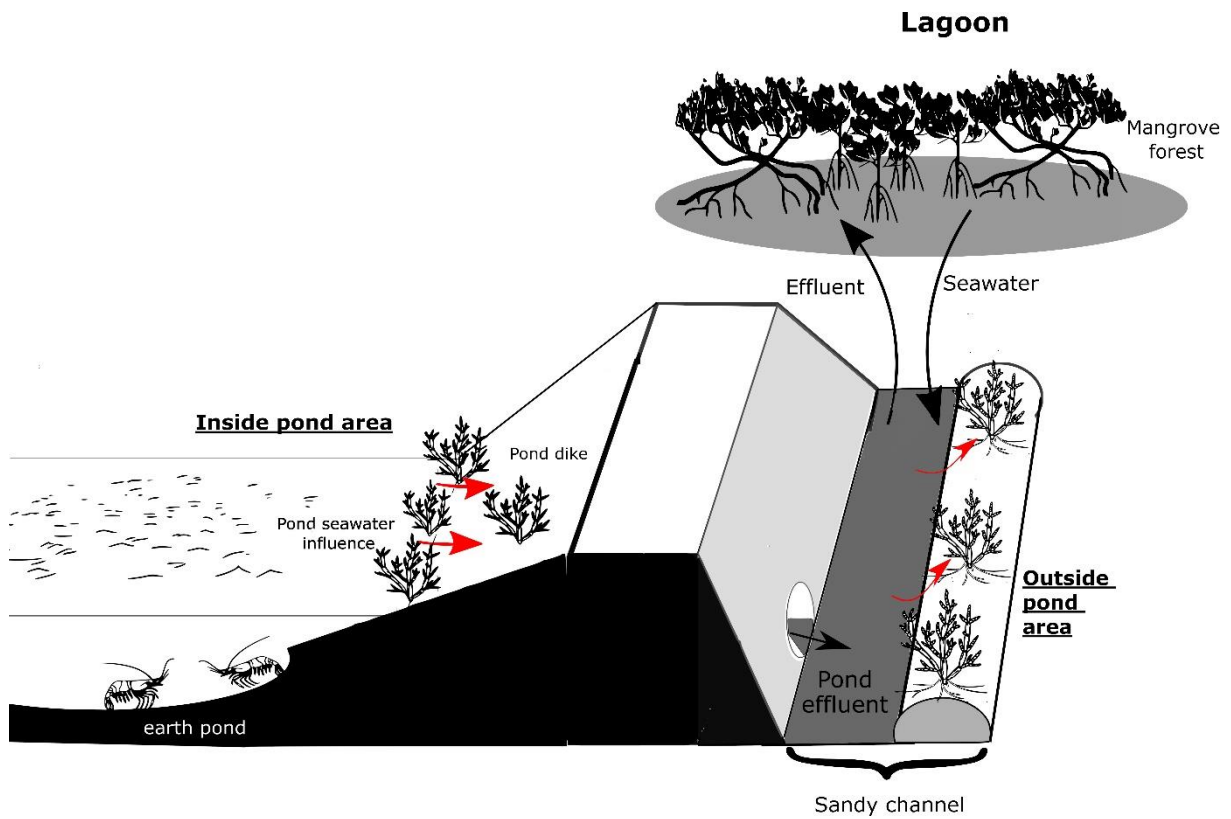


Figure 1. Schematic drawing of halophyte localization in shrimp farm. Red arrows symbolize the influence of pond water or shrimp effluents on halophyte growth.

3. Results

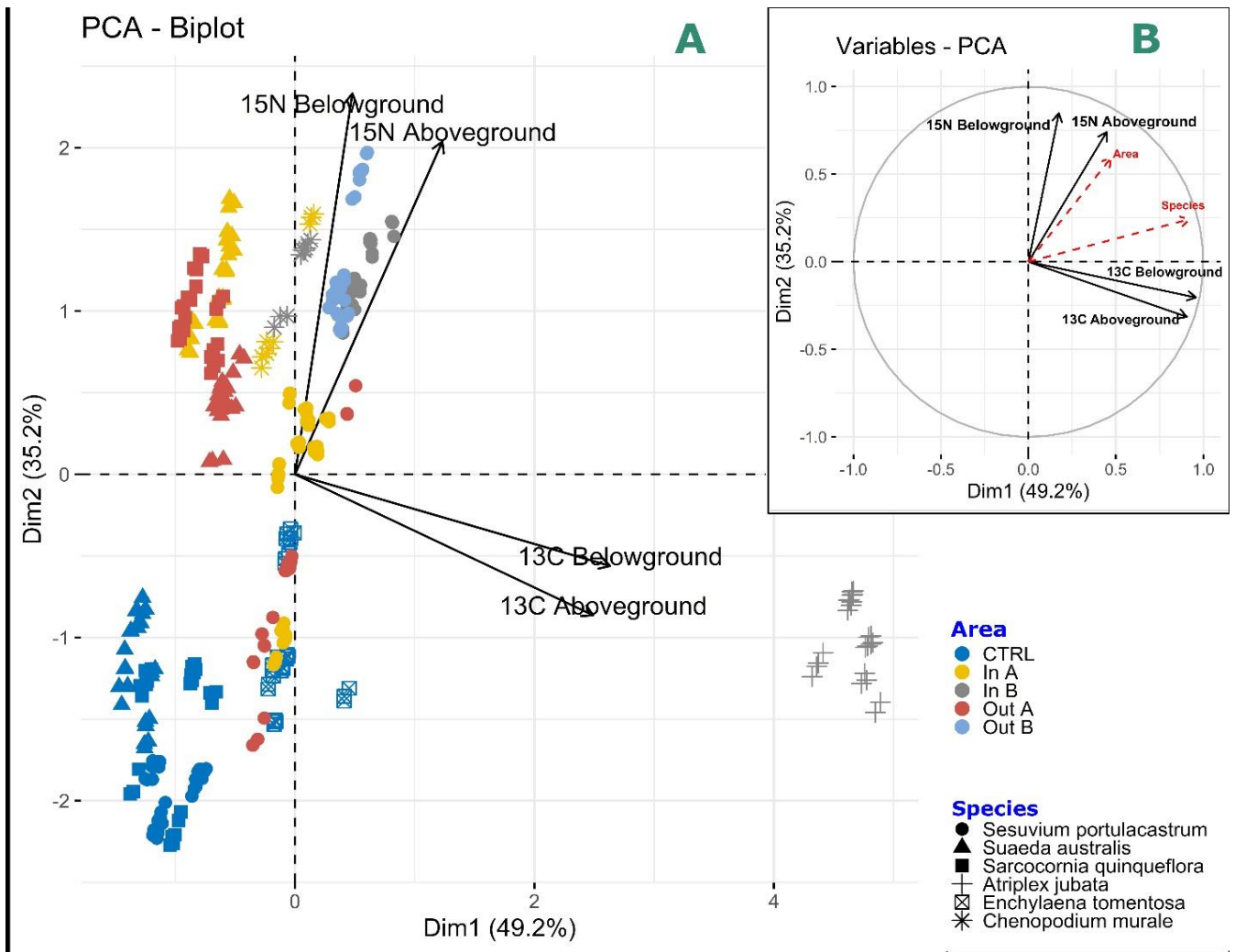


Figure 2: Principal component analysis (PCA). **A:** Biplot of sampled area and halophyte species based on the nitrogen and carbon isotopic signature of aboveground and belowground biomass. **B:** PCA indicating the relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of plant part following sampled area and halophyte species.

3.1.1 $\delta^{13}\text{C}$ isotopic signature in feed pellet, sediment and halophyte following sampled site.

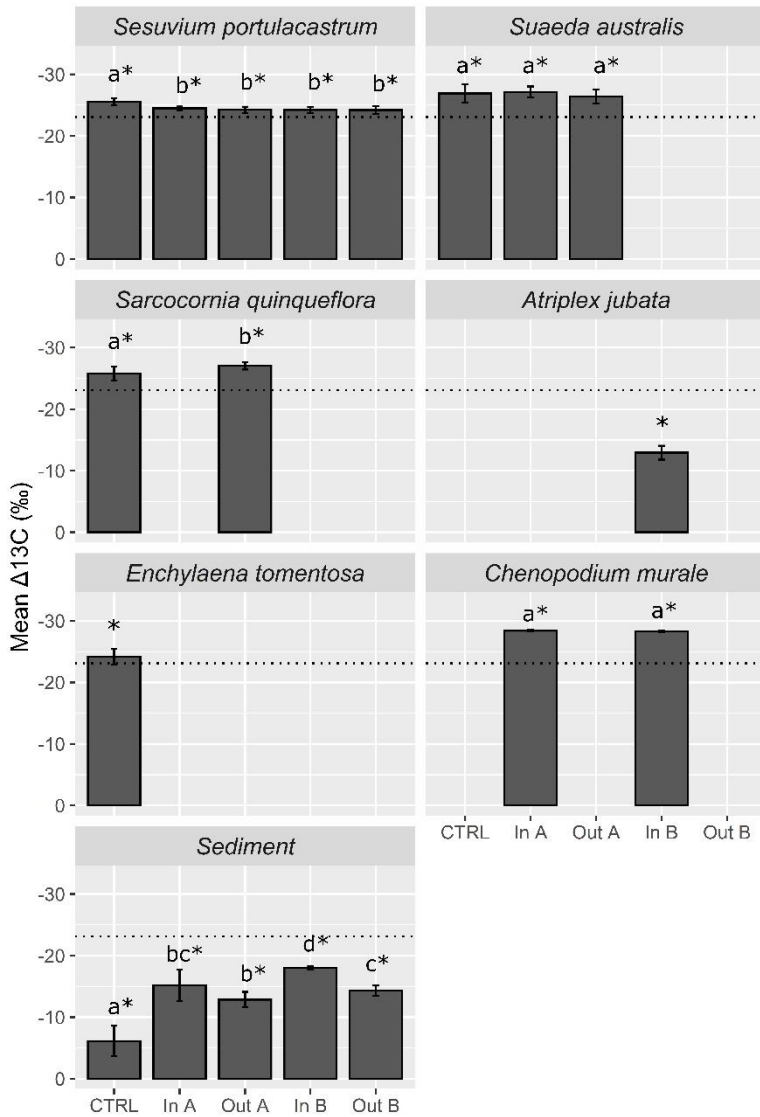


Figure 3. Means $\delta^{13}\text{C}$ values in halophyte biomass (aboveground and belowground) and sediment according to the sampled area (control, In and Out farm A and B) compared to feed pellet -23.08 ± 0.07 ‰ (dotted line).

« a, b, c, d », show significant variation between area and « * » statistical difference with the feed pellet.

3.2.1. $\delta^{15}\text{N}$ isotopic signature in feed pellet, sediment and halophyte following sampled site.

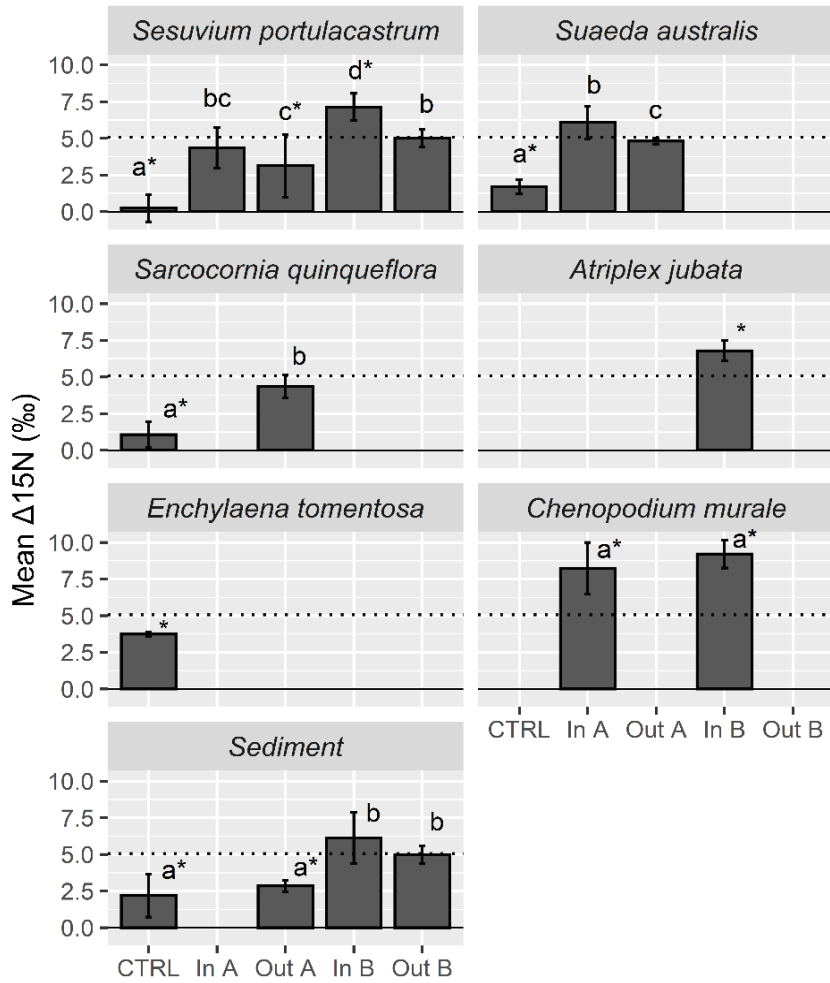


Figure 4. Means $\delta^{15}\text{N}$ values in aboveground biomass and sediment according to the sampled area (control, In and Out farm A and B) compared to feed pellet 5.06 ± 0.06 ‰ (dotted line).

« a, b, c, d », show significant variation between area and « * » statistical difference with the feed pellet.

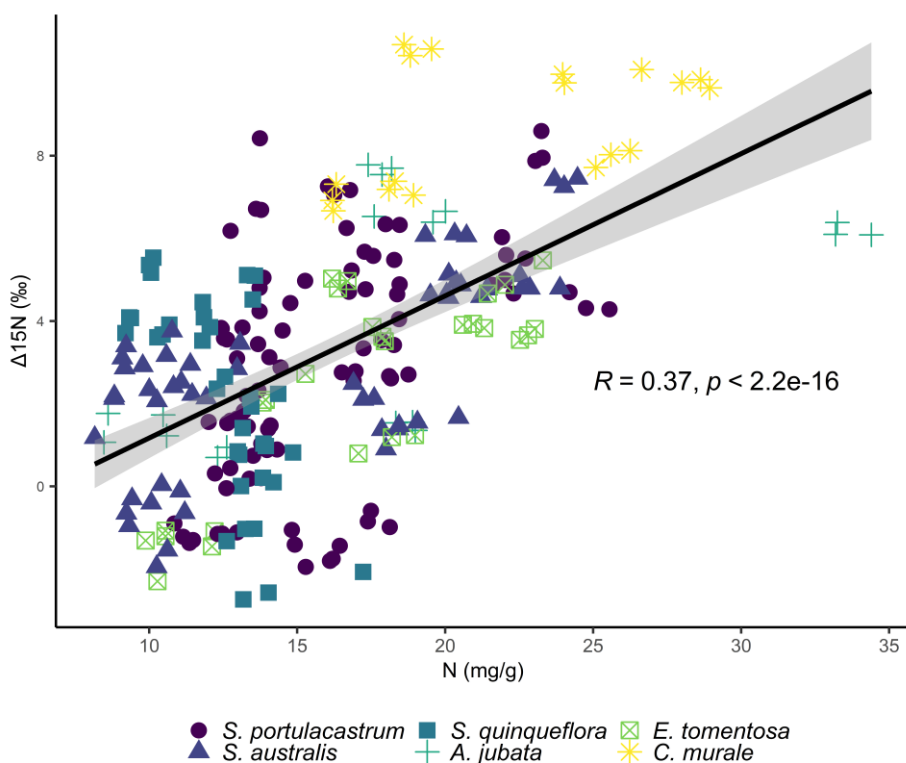


Figure 5. Correlation between $\delta^{15}\text{N}$ values and nitrogen content in whole halophyte biomass.