Mémoire de fin d'études

présenté pour l'obtention du diplôme d'Ingénieur Agronome
Option : Eau, Sol, Environnement

Evaluation of the root growth response of sugarcane/legume association under contrasting nitrogen and water availability

par Léa CHEVALIER

Année de soutenance : 2021

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Année de soutenance : 2021

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Résumé

Titre : Evaluation de la réponse de la croissance racinaire des associations canne-à-sucre / légumineuse sous disponibilité en azote et en eau contrastées.

La canne-à-sucre est une plante semi pérenne cultivée sur 22 700 ha à la Réunion. Elle est encore très dépendante des produits phytosanitaires tels que l'urée ou les désherbants chimiques. Dans le cadre du plan ECOPHYTO et de la réduction des herbicides, la réduction des intrants chimique au sein de la culture de la canne, est un levier majeur pour réduire l'indice de fréquence de traitement herbicide (IFTH). L'introduction de légumineuse en intercalaire de la canne-à-sucre permettant de contrôler les adventices et pouvant fixer l'azote atmosphérique pourrait limiter cette dépendance aux intrants. Néanmoins, peu d'études se sont intéressées aux processus sous-jacents de compétition entre les espèces, en particulier, le compartiment racinaire qui va être déterminant dans la répartition des ressources. Nous nous sommes donc proposés d'étudier la réponse racinaire de la canne à sucre en association avec des légumineuses sous disponibilité en azote et en eau contrastées. Nous avons pu observer que la présence de légumineuses semble impacter la densité de biomasse racinaire essentiellement dans le rang de canne plutôt que dans l'interrang. La densité racinaire quant à elle décroit avec la profondeur et avec l'augmentation de la distance au rang de canne que ce soit en pure ou en association. De manière générale, 80% des racines se trouvent dans les 30 premiers centimètres et plus de 50% des racines se trouvent au plus proche du rang de canne que ce soit en association ou en pure. Enfin la densité racinaire de canne est influencée par la disponibilité en azote et en eau. Il est possible d'observer une augmentation de la densité racinaire pour les traitements irrigués : 47% pour le traitement irrigué, non fertilisé et 27% pour le traitement irrigué, fertilisé. La tendance inverse est observée pour les racines de la plante de service avec +18% de densité racinaire pour le non irrigué et +51% de densité racinaire pour les modalités fertilisées. Au vu des résultats préliminaires il ne semble pas y avoir de séparation de niche racinaire entre la plante de service et la canne-à-sucre mais un effet de compétition sur la croissance de la canne.

Mots clés

Canne à sucre ; *Canavalia ensiformis* ; plantes de service ; distribution racinaire ; fertilisation ; irrigation.

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Abstract

Sugarcane is a semi-perennial crop cultivated on 22,700 ha in Reunion Island. It is still very dependent on phytosanitary products such as urea or herbicides. Within the framework of the ECOPHYTO plan and the reduction of herbicides, the reduction of chemicals inputs in sugarcane production is a major lever for reducing the herbicide treatment frequency index (HTFI). One solution is the introduction of leguminous as companion crop in sugarcane crops to control weeds and fix atmospheric nitrogen. However, few studies have been focused on the underlying processes of competition between species, in particular the root compartment, which will be a determining factor in the distribution of resources. We therefore proposed to study the root response of sugarcane in association with companion crops under contrasting nitrogen and water availability. We observed that the presence of companion crops impacts the root biomass density in the cane row rather than in the interrow. Root density decreased with depth and with increasing distance from the cane row, whether in pure or in association. In general, 80% of the roots were found in the first 30 cm and more than 50% of the roots were found as close as possible to the cane row, whether in association or pure. Finally, the root density of the cane is affected by the availability of nitrogen and water. It was possible to observe an increase in root density for the irrigated treatments: 47% for the irrigated, unfertilised treatment and 27% for the irrigated, fertilised treatment. The opposite trend was observed for the roots of the companion crop with +18% root density for the non-irrigated and +51% root density for the fertilized modalities. In view of the preliminary results, there did not seem to be any separation of root niches between the companion crop and the sugarcane but a competitive impact on cane growth.

Key words

Sugarcane; *Canavalia ensiformis*; companion crop; root distribution; fertilization; irrigation.

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Table of contents

Table of tables

Table of figures

Glossary

Companion crop: cultivated with the main crop and not harvested. It is expected to provide a service.

WinRhizo: software to analyse a root image and give root length and diameter.

Root to shoot ratio: root biomass ($T.ha^{-1}$) / aboveground biomass ($T.ha^{-1}$). With aboveground biomass stem and leaf.

Shoot biomass: aboveground biomass.

Abbreviations and acronyms

Cirad: Centre de coopération international en recherche agronomique pour le développement

IFTH: herbicide treatment frequency index

SALSA: Services ALloués aux Systèmes Agricoles

SD: Sowing Date

±W: Irrigation management

-W: Unirrigated

+N: Irrigated

±N: Nitrogen management

-N: Unfertilized

+N: Fertilized

±W±N: Irrigation and nitrogen management

±CC: Companion crop management

CAS: Pure sugarcane

CAS±CC: Association between sugarcane and companion crop

LAI: Leaf Area Index

1. Introduction:

Faced with new environmental and societal constraints, the agricultural sector must adapt to reduce the impact of intensive agriculture on the environment. In this context, crop diversification has a long history of research documenting benefits for farmers and the environment (Kocira et al., 2020). The use of cover crops or companion crops are being increasingly used in innovative cropping systems to favour biological regulation and to deliver agro-ecosystem services such as weed, pest or erosion control, as well as nitrogen fixation (Altieri et al., 2011; Koohafkan et al., 2012). Several types of associations exist i) cover crops, cultivated before or after the main crop to cover the soil (Vissoh et al., 1998), ii) companion crops, cultivated with the main crop and not harvested (Robertson, 2004) and iii) intercrops, cultivated with the main crop and harvested as a complementary cash or food crop (Vissoh et al., 1998).

Sugarcane (*Saccharum officinarum)* production represents a major source of sugar, ethanol and material for electric energy productions in tropical and sub-tropical regions. Nowadays, more than hundred countries grow sugarcane in a total area of 265,000 km². Demand is expected to increase, particularly for energy uses (ethanol, electricity) and the extraction of new molecules from biomass (Goldemberg et al., 2014; Leal et al., 2013) thus leading to an increase in surface areas (Van Ittersum et al., 2013). These areas will have to deal with the new production constraints that are emerging. In the case of sugarcane cultivation, several studies have shown the positive effects of crop association with other plants, such as corn (Pillay et al., 1978) or soybean (Li et al., 2013).

In France, the reduction of phytosanitary products has become a priority, leading to the implementation of a national herbicide and pesticide reduction program (ECOPHYTO, https://agriculture.gouv.fr/ecophyto) and the banning of numerous products such as glyphosate (ANSES). In Reunion Island, sugarcane farming, which represents 54% of the useful agricultural area (Agreste, 2019), must be adapted to these new societal and environmental constraints. Consequently, several alternatives to chemical inputs (herbicide and mineral fertilizer) are studied. The introduction of companion crop before planting or in the interrow aimed to control weed growth as an alternative to herbicide (Christina et al., 2021; Bhullar et al., 2006) and increase soil fertility through N-fixation, as an alternative to nitrogen fertilisers (Vandermeer, 1992; Viaud et al., 2020). As an example, in Reunion Island, the CanécoH project of the Dephy Expé network has shown that the introduction of companion crops, covering 80 to 90% of the inter-row, lead to a reduction in the herbicide treatment frequency index up to -70% (Mansuy, 2018).

However, the introduction of companion cops with sugarcane can lead to competition and yield losses. As an example, studies in Reunion Island have shown that the introduction of companion crops induced yield losses ranging from 16% with *Vigna Unguiculata* to 19% with *Canavalia ensiformis* (Mansuy et al., 2018). Yield loss could result from competition for resources. For example, previous studies in Brazil have shown that companion crop can lead to competition for water resources (Otto et al., 2020). Another study in Africa has shown that, depending on row spacing, competition for light can occur between sugarcane and companion crop (Singels et al., 2009). Finally, a study on sugarcane in association with soybeans have shown a lower access to N nitrogen fertilizer in the intercropping than in monoculture (Yang et al. 2013). Consequently, a trade-off should be found between yield reduction, weed control and improvement in soil N fertility.

The root compartment will be a determining factor in the allocation of water and nutrients resources between sugarcane and companion crops. To our knowledge, the response of the sugarcane root systems to the introduction of a companion crop has never been published in the scientific literature. Nonetheless, one study has been conducted in Reunion Island by eRcane and CIRAD during an internship (Kubinski, 2015). They observed an effect of the presence of jack beans *(Canavalia ensiformis)* on the sugarcane root distribution with a reduction in root density particularly in the inter-row in the first year of plantation. Such results suggest a possible root niche separation between sugarcane and companion crop. Nevertheless, these results in plantation, fertilised and irrigated, are likely to evolve according to the stage of the cane (plant vs ratoon crop), as well as according to the resources availability (e.g. water and fertiliser).

In a recent CIRAD trial in Reunion Island (Viaud et al., 2020) where jack bean was tested as a companion crop in a ratoon sugarcane, a trend to decrease in root biomass at harvest was observed in the interrow where jack beans was present. This slight decrease could result from an absence of root niche separation in ratoon crop and/or a recolonisation of cane roots in the inter-row after the end of the jack bean cycle. In addition, irrigation had an influence on the root biomass of jack bean in this trial and therefore could influence the distribution of cane and legume roots. To confirm or not the hypothesis of a root niche separation, further studies are required.

2. Purpose and research questions:

The objective of this internship was to evaluate the root growth response of sugarcane/legume associations under contrasting levels of nitrogen and water availability.

The specific research questions were:

- What is the effect of the introduction of jack bean on cane root biomass, distribution, and architecture (diameters and lengths)?
- How do nitrogen and water availability influence sugarcane and legume roots in association?
- Is the separation of cane and jack bean root niches observed in plant crop is also valid in ratoon crop?

3. Context

3.1. Reunion island

Reunion Island is a volcanic located in the Indian Ocean in the Mascarene Archipelago about 684km from the east coast of Madagascar, and 172km west from Mauritius. The highest point in the island is the Piton des Neiges, which rises to 3,071m. The topography is steep with numerous valleys and the island's hydrographic network is well developed, leading to pronounced erosion. The island has a still active volcano: the Piton de la Fournaise located in the south-east. The last eruption was on 9 April 2021.

Reunion island has two distinct climatic zones: the “windward coast” and the “leeward coast”. The mountains in the middle of the island block the winds and clouds coming from the east. This part of the island is therefore very humid, whereas the western zone is drier (Fig. 1). However, the tormented relief of the island creates various microclimate zones. Thus, strong rainfall gradients can be observed, from 600 mm to 9,000 mm.y^{-1} (Fig. 1). The difference between the western and eastern zones of the island is marked by the isohyet of 2000 mm. In the west, the maximum annual rainfall observed is 2000 mm y^{-1}, whereas in the east it is 9000 mm y^{-1}.

Two seasons can be observed: summer and winter. Summer is marked by high temperatures and high humidity caused by torrential rains in the highlands. It lasts from January to March and the air temperature can reach 35°C. Monthly rainfall on the leeward side (west coast) ranges from 100 mm to 300 mm and on the windward side (east coast) from 200 mm to 900 mm of rain per month. Winter is characterised by a temperature of 25°C on the coast and 19°C in the interior of the island and runs from May to November. This season is also called the dry season, as rainfall is rare. On the west coast (leeward) about 100 mm of water falls per month compared to 100-300 mm on the east coast (windward). April and December are transitional months which can be very rainy or very dry. Cyclonic episodes are also observed. They occur mainly during the summer (http://www.meteofrance.re/).

In general, temperatures vary according to the season, altitude and wind exposure. The west leeward region is generally 1 or 2°C warmer than the east windward region (Raunet, 1991). It is common for the temperature to reach 0°C on the summits.

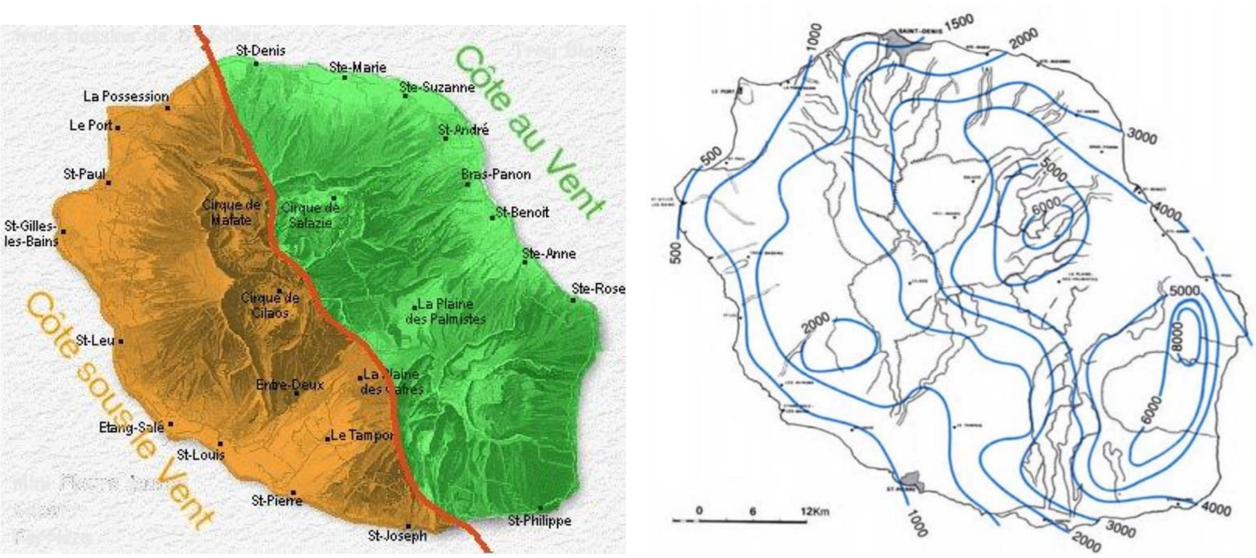

Fig. 1. Exposure to prevailing wind (Left) and rainfall map of Reunion Island (Right), source L'île intense, 2017.

Reunion Island is a "hot-spot", so the parent rock is volcanic. Volcanism is predominantly effusive (lava flows), but it has been possible to observe explosive episodes in the past (clouds, ashes, tuffs, etc.). The geology is diverse in terms of lava chemistry, emission types and chronology.

The soils formed on the island are the result of the influence of time, climate, biological agents, and topography on the parent rock. The variability of the parent rock and the contrasting climate of the island therefore lead to the formation of diversified soil types (BD Topo 2PP3 IGN, pedological data CIRAD_Région Réunion 1991). It is possible to observe a stratification of soils around the island according to altitude and climate:

- Ferralitic soils / Nitisols.

These soils are derived from the hydrolysis of volcanic rock minerals. These are the oldest soils and the less numerous because they are located on the eastern side of the island. This face is the most subject to violent climatic episodes: rainfall and winds, erosion is therefore very marked, and some materials have been completely eroded.

- Brown soils (below 400 m altitude) / cambisols
- Andic brown soils (from 350 to 600 m altitude) / Andic cambisols
- Vertisols (below 400 m altitude) / Vertisols

Brown soils and Vertisols are formations resulting from the influence of a dry climate with little vegetation cover.

- Non-Perhydrated Andosols (from 600 to 1300 m altitude) / Andosols
- Perhydrated Andosols (from 1300 to 1600 m altitude)

Andosols are young soils, derived from recent pyroclastic lava flows. They are known for their high fertility and water retention capacity, which allow high production rates.

3.1. Sugarcane in Reunion Island

The variability of the island's climatic and geological factors has influenced the agriculture that has been developed. From 1815 onwards, Reunion Island turned to the cultivation of sugarcane, which replaced coffee, which was considered too sensitive to cyclonic risks. At that time, sugarcane was grown from the sea to the mountains and covered 60,000 hectares. Today, sugarcane is grown on 22,700 hectares (54% of the UAA). It remains the most widespread crop on the island (Agreste, 2019). Sugarcane has an important economic and environmental role in the island. In particular, sugarcane plantations limit soil erosion due to its rapid ground cover and its strong root development.

Sugarcane cultivation is subject to several constraints such as the development of bio-aggressors (including white grubs, rats and weeds), low temperatures in high altitude as well as drought and cyclones. Several of these constraints have been reduced through breeding, with the selection of cane varieties resistant to pests, or through biological control of white grubs. Weeds are currently the main factor in yield loss. Indeed, yield loss reaches 15 t ha^{-1} for one month of weeding delay, representing 200 kg ha^{-1} per day of weeding delay (Marnotte et al., 2008).

Weed control is mainly based on the use of herbicides. However, alternative methods to herbicides have been tried and tested, such as mechanical weeding of the inter-row, straw tedding, thickening or manual weeding (Ecophyto, 2016). These methods of control do not allow a total control of weeds and are often complementary to the use of herbicides.

With the implementation in 2018 of the Ecophyto II+ plan, the reduction in phytopharmaceutical products has become a strong public expectation and is seen as a necessity to preserve human health and biodiversity (Ministère de l'agriculture et de l'alimentation, 2021). The main objectives of this plan is "an agriculture less dependent on pesticides" and the "glyphosate exit plan" announced on 22 June 2018 (DAAF). In Reunion Island, the herbicide treatment frequency index (IFTH) for sugarcane decreased by 44% between 2010 and 2014 (DAAF, CROS, 2016). In 2020, the sugarcane IFTH was 3.1 (Agreste, DAAF, 2020) one of the lowest IFTH in comparison to other crops. Nonetheless, as sugarcane covers 22,700 ha, making it the most common crop in the island (Agreste, 2019), sugarcane was still the most pesticide consuming crop (Agreste, DAAF, 2020). In addition, with the removal of some key herbicides (e.g. glyphosate, ANSES, 2021), it has become essential to find alternative practices that reduce or even eliminate the use of herbicides.

In a perspective of sustainable agriculture, it is also necessary to reduce the use of mineral fertilisers or improve its efficiency. As sugarcane fertilisation is essential, but costly (Building, 2008), optimising fertilisation is important to reduce the environmental impact (e.g. nitrate lixiviation) while maintaining the profitability of the crop for growers. Nitrogen is considered the main limiting factor of sugarcane in Reunion Island. Nitrogen promotes the growth of sugarcane by activating photosynthesis (Caro Canne n°32, 2014) as well as the level of absorption of other nutrients (Chabalier, 2020). Yet, several analyses highlight the fact that globally high annual inputs of nitrogen in agricultural systems cannot be carried out without causing significant damage to the environment (FAO, 2015). Limiting the nitrate content in infiltration water is a major environmental concern, as the increase in this element in watercourses favours the eutrophication of the environment (Toussaint, 2010). Studies of alternative fertilisation to mineral fertilisation have already been carried out by eRcane and CIRAD, in particular with the use of organic fertilisers or N-fixing species to reduce the use of synthetic fertilisers.

3.2. Sugarcane life cycle

Sugarcane is a giant perennial grass of the grass family. It can grow back several years without being replanted, after each harvest. The CTICS of Reunion Island advises a duration of 7 years between each planting episode. However, the average length of time applied between each plantation in most farms on the island is around 10 years.

During the growth, the sugarcane accumulates sugar in the stalk until it reaches the stage of maturity (before flowering) when the plant is ready to be harvested. It is possible to distinguish 5 stages until harvest (Fillols & Chabalier, 2007, guide de la fertilisation):

- The emergence after few weeks, which is done from the cutting (plant crop) or stumps (ratoon crops). It takes a few weeks.
- The tillering phase, which follows emergence. During this phase tufts of stems are formed in underground buds from the primary stems. Tillering phase is influenced by temperature conditions, water excess or stress and nutrition.
- Growth, which is the stage of leaf development, cane elongation and root development. The leaves appear every one to three weeks. The root system of the cane is adventitious branched and can go down to 6m deep depending on soil depth. 90% of the roots are concentrated in the first 60 cm of the soil (Fauconnier, 1993; Fillols & Chabalier, 2007)
- Maturation where the sugar is stored as saccharose in the cane stalk. Sugar storage starts at the beginning of growth and increases during the winter under stressful conditions (water stress, nitrogen deficiency, higher diurnal range temperature).
- Flowering is characterised by the transformation of the apical bud into a floral bud that will later give rise to an inflorescence called an "arrow". Flowering depends on climatic conditions and varieties.

If the cane is not harvested immediately, it is possible to observe a phase of over-maturation which corresponds to a remobilisation of sugars and a resumption of growth.

The stages occur at different time depending on temperature in each climatic zone in the island as well as the altitude (Poser, 2013). Sugarcane can reach between 2 and 4 metres in height, depending on the variety. Sugarcane is a water-consuming crop, with water requirements reaching 1,000 to 1,700 m^3/ha/month in periods of strong growth. Therefore, the West part of the Island requires irrigation (particularly in low altitude) to grow sugarcane.

3.3. Intercropping in Reunion Island

Crop association consists in cultivating several plant species or varieties in the same plot at the same time (Ziberlin, 2010). In Reunion Island, the DAAF (Direction de l'Alimentation, de l'Agriculture et de la Forêt) recommends crops associations for different reasons. For example, the use of cover crops is recommended to limit soil erosion between two crop cycles or in arboriculture systems to diversify production. Another example is to increase crop protection against pests and diseases.

In the aim of crop protection against pests, various practices have been studied and are used by farmers. For example, in 2009, following a study of fruit fly control methods, CIRAD showed that by combining Cucurbitaceae plantations with maize or sugarcane, it was possible to reduce the impact of pests on this vegetable crop. Indeed, borders of maize or sugarcane create a mosaic of landscapes and attract pests. It is therefore possible to treat these plants with traps or phytosanitary products without directly treating the Cucurbitaceae (Ryckewaert, 2009).

Additionally, well-chosen companion crops to limit weed growth and the use of N-fixing species make it possible to reduce the use of chemical inputs. The introduction of these crops in sugarcane aim to provide several ecosystem services: weed control, soil fertility, erosion protection, insect control, etc. Companion crops, especially legumes, could thus become a key element of a more sustainable agriculture, integrating the minimisation of production costs and the growing environmental challenge

(Vertes *et al*., 2010).

Because of the benefits of crop association in agricultural sectors in Réunion Island, CIRAD and eRcane have set up trials to evaluate the effect of companion crop in sugarcane. Several projects can be cited: MAGECAR 2010-2012, Ecocanne 2012-2016, CanécoH 2013-2018. In total, about ten species have been tested as companion crops with sugarcane. Thanks to these trials and a study carried out between 2016 and 2018 to determine the most effective plants for weed control. It was possible to show that the most productive companion crops limited weed growth to less than 30% of the plot (Christina et al., 2018). In particular, the jack bean has been highlighted as a particularly effective companion crop in association with sugarcane.

Recently, in 2018, a new trial was set up by CIRAD to assess the ability of legumes in association with sugarcane to improve soil fertility and resource use efficiency between the two species under contrasting nitrogen and water availability. This trial will be the focus of the study in this document and the leguminous used was the jack bean (*Canavalia ensiformis).*

3.4. Jack bean life cycle

Jack bean (*Canavalia ensiformis*) is an annual legume. It is believed to be native from Central America and tolerates drought, shade, moderate flooding and saline soils (ADAR, 1994). It can grow up to 1800 m altitude in places where rainfall varies between 600 and 2000 mm.

Jack bean develops slowly in the early stages of growth and appears as a woody vine or shrub that can grow up to 1 m (CTCS, 2015). The leaves are trifoliate and can reach 20 cm in length and 10 cm in width. The flowers are pink, purple or white with a red base and are about 2.5 cm long. The pod is sword-shaped and can reach 40 cm, the seeds are white and smooth. Each pod contains 10 to 14 seeds (CTCS, 2015). The jack bean can be distinguished in several stages of development (CTCS, 2015), their total development last 1,800 degrees days (Trail et al., 2019):

- Seed germination. This stage takes place 2 to 3 days after sowing, at temperatures between 19 and 43 °C.
- Jack bean flowering is divided into three periods: early flowering about 58 days after sowing, mid-flowering about 84 days after sowing and late flowering about 90 to 110 days after sowing (USDAS, 2013).
- Pods are produced after 80-120 days after sowing and the ripening phase lasts between 180-300 days after planting (USDA, 2013).

Jack bean is being studied in association with sugarcane to control weeds by limiting their development through rapid soil cover and to improve soil fertility (Fig. 2). According to INRA, jack bean in pure stand could provide a potential of 800 kg of nitrogen for 20 tons of dry matter per hectare and per 9-months cycle.

Fig. 2. Sugarcane in association with Jack bean (left) or without (right) in a ratoon crop in CIRAD (personal photography, 2021).

4. Material & Methods:

4.1. Experimental designs

Measurements were carried out in two experimental trials located in the experimental station of La Mare (Reunion Island). The first trial (SALSA trial – "Services alloués aux systèmes agricoles") was located at coordinates -20.903224, 55.531035 and the second trial (Sowing-date trial) was located nearby (-20.902139, 55.529652).

4.1.1. SALSA trial

The objective of the SALSA trial was to assess the ability of jack bean in the interrow of sugarcane (during planting and ratoon) to improve soil fertility and resource use efficiency under contrasting levels of nitrogen and water availability. The trial was planted in October 2018 with 4 blocks and 32 elementary plots (Fig. 3), so the ratoon crop was studied in this internship. Each elementary plot consisted of 8 rows of cane 11 m long and 1.5 m interrow. Three treatments and their combinations were assessed in the trial. The trial was divided into two zones with 16 non-irrigated plots (-W) and 16 irrigated plots (+W). In each zone, two treatments were randomized: a treatment with (+N) or without (-N) urea application and a second treatment with jack bean as companion crop (+CC) or not (-CC).

- Irrigation management: in +W, plots were irrigated twice a week during 3 hours, for an average annual irrigation of 900 mm. In -W, plots were irrigated only the two first months of growth.

- Fertilisation management: plot was fertilized manually in the row with P (42 kg/ha) and K (190 kg/ha) after harvest. Considering urea fertilizers, it was split into two equal applications (68.5 kg/ha x 2) one month and 5 months after harvest. The sugarcane was fertilised to meet 100% of its needs (Table 1).
- Companion crop management: in +CC treatments, jack beans were sown manually 1.5 month after harvest on two rows distanced from 40 cm from each other and 55 cm from the cane row (Table 1). Seeds were distanced by 40 cm from each other on the legume row. This means that the bean was located 55 cm from the cane row. The seeds were sown at a density of 58 kg/ha under the cane mulch. The bean was left for the entire 12-months cane cycle, but naturally died around 7 to 9 months after harvest.

Block number 4 did not react well during planting, so roots were sampled only in the first three blocks. The sampling was therefore performed in 24 plots with 3 repetitions of each modality combinations.

The sugarcane and legume management (harvest and sowing dates) are detailed in Table 1.

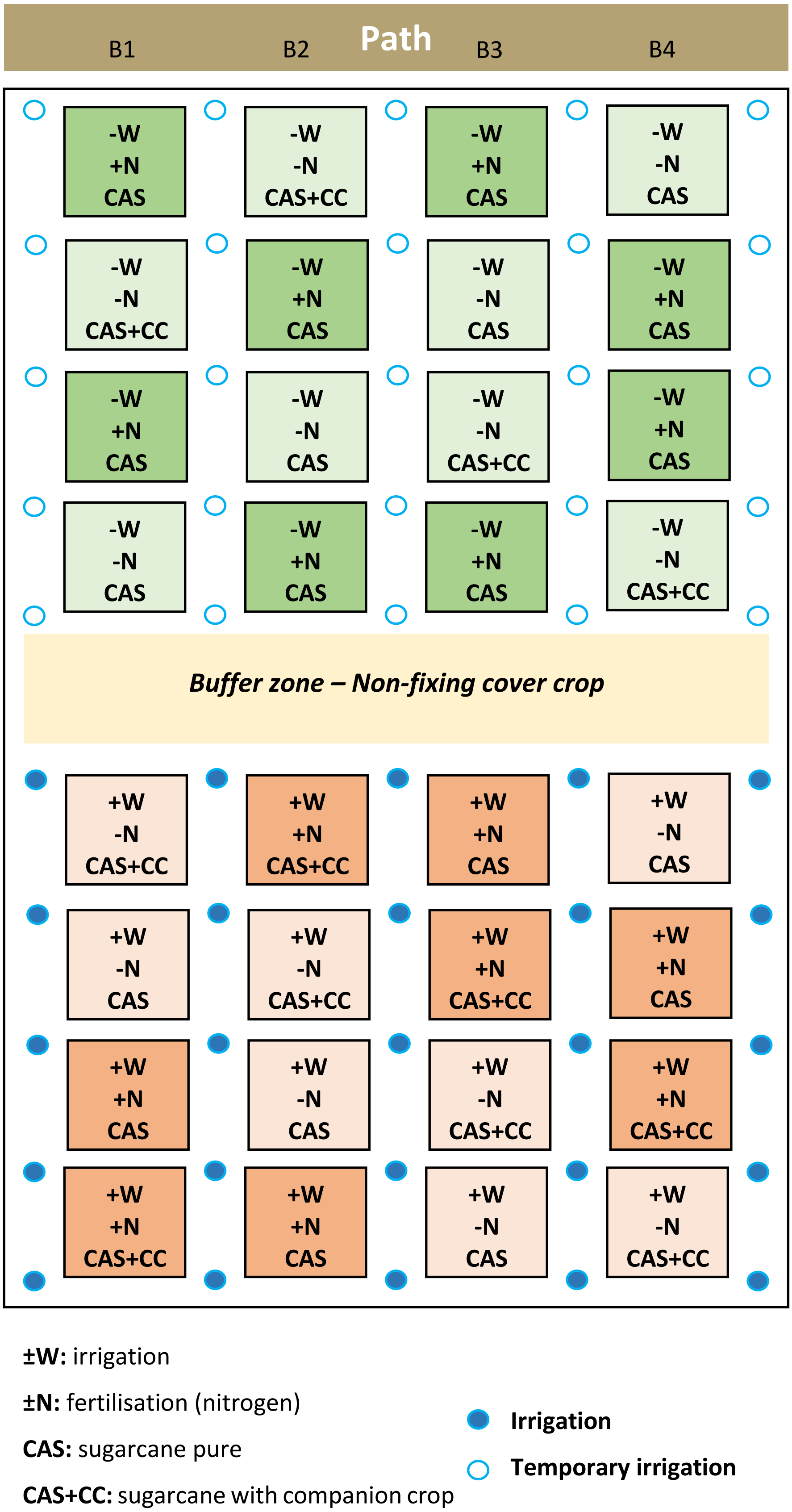

Fig. 3. Experimental design of the Salsa trial.

4.1.2. Sowing date trial

The sowing date trial (Fig. 4) consisted of pure jack bean plots with the same treatments and combinations than the SALSA trials: irrigated (+W) or non-irrigated (-W), fertilised with urea (+N) or not (-N). Each modality combination was repeated three times for a total of 12 plots. The trial was sown manually in November at the same time as the jack bean sown in the Salsa trial and fertilisation was applied after sowing, with P (35 kg/ha), K (160 kg/ha). For the plots which were fertilised with nitrogen, 68.5 kgN/ha at sowing was applied corresponding to the second fertilization in the SALSA trial (Table 1). The jack bean plants were sown in lines with 40cm interrow. Seeds were distanced by 40 cm from each other on the legume row.

Fig. 4. One plot of SD trial (Mathias Christina photography, 2021)

4.1.3. Trial comparisons

In the study we have three different cropping systems: pure sugarcane (CAS), sugarcane with companion crop (CAS+CC) and pure companion crop (CC) combined with four nitrogen and water availability treatments (+W+N, -W+N, -W-N, +W-N). Each treatment combination was repeated three times. While CAS+CC and CAS cropping systems can be directly compared because there were tested in the same trial, the CC cropping systems was tested in other trials with a slightly different type of soil (Table 1), located around 100 m from the SALSA trial.

Even though the trials are only a few hundred meters apart, it was possible to observe differences in the soil analyses (Table 1). One difference to be observed is that of pH, which ranges from 6.1 at the surface in Salsa to 6.45 at depth, whereas in SD the values range from 5.8 at the surface for +W to 6.03 at the surface for -W. It was also possible to see differences in nutrient availability for mineral nitrogen with 124.48 kg/ha/yr for Salsa against 130.77 kg/ha/yr and 109.32 kg/ha/yr for +W and -W respectively. The differences are even marked between the two modalities +W and -W of SD. The biggest differences to be observed are the amount of phosphorus with 115.7 mg/kgDM soil for Salsa and 22.96 mg/kgDM soil and 19.72 mg/kgDM soil for +W and -W, respectively. As these parameters could influence plant growth and the soils were different, it will be difficult to compare the root response of jack bean between the two trials and disentangle the sugarcane effect from soil on legume growth.

The trial management were similar in both treatments, in terms of fertilisation, CC sowing date, manual sowing method and density (Table 1). The jack bean was sown on the Salsa trial 2 months after harvest, on 15 December. The Sowing-date trial was sown at the same time in order to have a pure control in jack bean in the same climatic zone. On both plots the sowing was performed manually.

Table 1. Crop management and soil characteristics in both trials.

Management		Salsa trial	Sowing-date trial	
Sugarcane harvest date		October		
Jack bean sowing date		14-17/12/2021	14-17/12/2021	
Fertilization treatment	N	68,5 kg/ha x 2 (cane harvest and legume sowing)	68,5 kg/ha	
	P	42 kg/ha	35 kg/ha	
	K	190 kg/ha	160 kg/ha	
Jack sowing density		58 kg/ha	50 kg/ha	
Soil characteristics				
Soil type (name)		Nitisol		
			+W	-W
Clay %	0-20 cm	16.39		
	20-40 cm	17.68		
	40-60 cm	55.64		
Silt %	0-20 cm	54.9		
	20-40 cm	55.62	-	-
	40-60 cm	34.27		
Sand %	0-20 cm	28.71		
	20-40 cm	26.7		
	40-60 cm	10.09		
pH	0-20 cm	6.1	5.8	6.03
	20-40 cm	6.25		
	40-60 cm	6.41		
Bulk density (g soil/cm3)	0-20 cm	1.16		
	20-40 cm	1.29		
	40-60 cm	1.15		
Field capacity cm3/cm3	0-20 cm	38.012		
	20-40 cm	48.480	-	-
	40-60 cm	40.807		
Permanent wilting point (v/v.)	0-20 cm	26.244		
	20-40 cm	27.002		
	40-60 cm	26.855		
Soil organic matter content (%)	0-20 cm	3.089		
	20-40 cm	2.890		
	40-60 cm	1.295		
N (g/kgDM soil)	0-20 cm	1.92	2.02	1.80
	20-40 cm	1.36	-	-
	40-60 cm	0.73		
N min (kg/ha/yr)	0-20 cm	124.48	130.77	109.32
	20-40 cm	88.19	-	-
	40-60 cm	47.37		
C (g/kgDM soil)	0-20 cm	21.33	22.96	19.72
	20-40 cm	14.2	-	-
	40-60 cm	7.34		
C/N	0-20 cm	11.1	11.38	11.69
	20-40 cm	10.43	-	-
	40-60 cm	10.04		
P (mg/kgDM soil)	0-20 cm	115.7	41.08	21.9
	20-40 cm	61.24	-	-
	40-60 cm	51.81		
K (cmol+ / kgDM soil)	0-20 cm	0.67	0.24	0.2
	20-40 cm	0.14	-	-
	40-60 cm	0.05		
Ca (cmol+/kgDM soil)	0-20 cm	6.46	6.28	6.03
	20-40 cm	5.37	-	-
	40-60 cm	4.28		
Mg (cmol+/kgDM soil)	0-20 cm	3.1	3.51	3.58
	20-40 cm	2.48	-	-
	40-60 cm	2.23		
Na (cmol+/kgDM soil)	0-20 cm	0.13	0.18	0.22
	20-40 cm	0.34	-	-
	40-60 cm	0.56		
S base (cmol+/kgDM soil)	0-20 cm	10.36	10.22	10.03
	20-40 cm	8.32	-	-
	40-60 cm	7.12		
Cation Exchange capacity [CEC] (cmol/kg)	0-20 cm	12.16	2.2	1.9
	20-40 cm	10.88	-	-
	40-60 cm	9.43		

4.2. Field sampling

In each trial, two types of samplings were carried out: mechanical auger sampling and manual excavation through ring sampling. The measurements were performed during three weeks from 10 March 2021 to 31 March 2021, corresponding to a cane of 6 months and a jack bean of 3 months.

4.2.1. Mechanical auger

In each of the three plot repetitions in the eight treatments of the SALSA trial (CAS vs CAS+CC combined with +W+N, -W+N, +W-N, -W-N), three samples were taken in the inter-row at three distances from the cane line: in the row (12.5 cm from the row), in the interrow (37.5 cm from the row) and between both (62.5 cm from the row, Fig. 6). The samples were taken using a Cobra TT thermal core sampler fitted with a 50 cm long auger with an internal diameter of 9 cm (Fig. 5). Samples were then split into 3 horizons, from 0 to 10 cm, 10 to 30 cm and 30 to 50 cm. Each sample was stored in a cold room at 4°C before being processed as quickly as possible. In total, 72 cores were sampled in the SALSA trial (8 treatments x 3 repetitions x 3 cores).

Similarly, in each of the three-plot repetitions in the four treatments in the sowing date trial (CC ±W±N), two samples were taken in the legume row and in the middle between two row (20 cm from the row). In total, 24 cores were sampled in the sowing date trial (4 treatment x 3 repetitions x 2 cores). Combining both trials and the different depths, 288 root samples were processed further.

Fig. 5. Field sampling mechanical auger (personal photography, 2021)

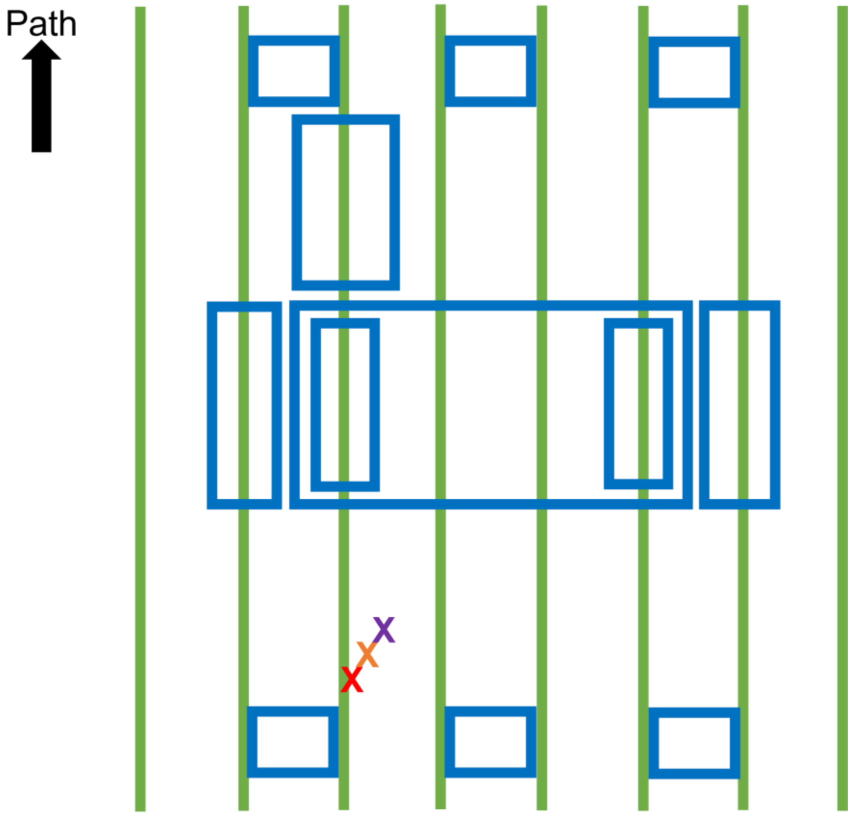

Fig. 6. Diagrams of root sampling zones in Salsa trial.

: Row

: Other's sampling (LAI and SWC measurements, inventory of cane diameter and height, harvest of cane, companion crop, mulch and weeds)

X: sampling point at approximatively 12.5 cm from the row

X: sampling point at approximatively 37.5 cm from the row

X: sampling point at approximatively 62.5 cm from the row

4.2.2. Manual excavation

As the jack bean has a taproot system, it was possible to miss the taproot with the auger. Consequently, we have added a complementary measurement called the circle method in order to sample the pivot root for the root biomass measurement. In each +CC plot, a 20 cm diameter PVC circle was placed around a jack bean. The aerial biomass of this individual was measured to carry out a ratio of aerial biomass to root biomass. Using a knife, the soil and roots from 0 to 10 cm and from 10 to 30 cm were sampled using the 20 cm diameter circle. Before being processed, the samples were stored in a cold room at 4°C. Similarly, a sample was taken in each -CC plot in the study as a sugarcane control. We therefore made 24 holes on Salsa trial and 12 holes on Sowing-date trial. With two depths, 72 additional samples were processed. The data obtained from the circle have not been presented in this report.

4.3. Laboratory measurements

4.3.1. Root extraction and washing

The sampling treatments begins with root extraction and washing. The method was different depending if the sample came from a mechanical auger or a circle.

For auger samples, the treatment consisted of successive washings of the soil samples to recover as many roots as possible. The roots floated on the surface of the buckets and were collected using 50 µm sieves. They were then separated from the organic matter and placed in airtight plastic bags to prevent them from drying out before being scanned. For mixed samples, jack bean roots and sugarcane roots were separated and kept in separate bags at the time of washing. The jack bean roots were less branched and lighter in colour than the sugarcane roots, making it possible to distinguish them.

For circle samples, as the volume of soil collected was very large, the total weight sample was measured. Then the soil was placed in a large plastic tray to collect the most visible roots, which were rinsed and bagged. The remaining soil was mixed and ¼ of the soil was collected and weighed. This soil then undergoes the washing process described above. The total amount of root in the initial sample was then calculated through the fresh weight proportion of the sub-sample in comparison to the total sample. Sugarcane and jack bean roots were separated before bagging and stored in the same way as the auger samples.

4.3.2. Root length and diameter calculation

The fresh roots were scanned to assess root length and diameter. The scanner used was the Epson perfection V850 Pro. This scanner allowed to scan up and down at the same time and thus to obtain high quality images of roots which could be used afterwards in the WinRhizo software.

All samples from the washing were scanned. The root samples were visually separated into two categories: larger than 1 mm diameter and smaller than 1 mm diameter (defining the limits between coarse and fine roots). They were then placed in a transparent petri dish filled with water to be scanned. The images were taken using the professional / colour positive mode with a 600-dpi resolution. The scanned images were then analysed in WinRhizo to obtain the total length and the mean diameter of the roots in each sample. During this internship, not all scans have been analysed yet. Therefore, the results on the lengths and diameters will not be presented.

4.3.3. Dry mass

All biomass samples (aerial jack bean in the circle sample; roots in the circle and auger samples) were dried in an oven at 60°C for 72 hours to obtain their dry masses. Root biomass density was then calculated as the total root dry mass per species divided by the volume of the auger or circle sample. Specific root length (SRL) was calculated as the total root length divided by the root dry mass.

4.4. Complementary results

Additional results obtained during a thesis (Pauline Viaud's Thesis) being carried out at the same time as this internship were also used. The aboveground biomass of sugarcane was estimated in each plot using the allometric relationship method between height and biomass of sugarcane (Poultney et al., 2020). Forwards, aboveground dry mass will be referred as shoot dry mass and the ratio with belowground biomass will be referred as root to shoot ratio.

4.5. Data analyses

The variables analysed in this study were: the total root biomass (g m^{-2}), the root density in each soil layer (g dm^{-3}), the shoot biomass (t ha^{-1}) and the root to shoot ratio. The influence of CC, W, N and their interaction on sugarcane total root biomass, shoot biomass and the root to shoot ratio was tested using a linear analysis of variance (ANOVA) without random effect. The influence of CC, distance from the row, soil depth and the combined "±W±N" factor and their interaction on root density were tested using a mixt linear analysis of variance with the core sample identification as random effect (nlme package; Pinheiro et al., 2019) in order to consider the dependence of the three depths in the same core. The root density was transformed using the BCOX function (mass package; Venables et al., 2002) before the variance analysis. Pairwise comparisons were performed using t test. Significant differences are represented by stars on the graphs with * for $p<0.05$, ** for $p<0.01$, *** for $p<0.001$ and for non-significative correlation ns. To represent the data, the figures were made with the package ggplot2 (Wickham, 2016). Data were processed using the R free software (version 3.6.2, 2019-12-12).

5. Results

5.1. Impact of the introduction of jack bean on cane root dry mass, shoot dry mass and root to shoot ratio

Sugarcane cane root biomass was significantly influenced by W management but not by CC and N management (Table 2). Root biomass was 39% higher in +W than in -W (Fig. 7a). Considering the p-value of 0.0766 from the CC effect on root biomass (Table 2), it seems possible to saw a trend of the effect of jack bean on the root biomass. The total root biomass of sugarcane has seemed to be 21% lower in the presence of jack bean than in absence, in average. Sugarcane root biomass were 1.8 and 2.3 t ha^{-1} in the presence and absence of jack bean, respectively. The impact of jack bean was particularly pronounced in the -W+N treatment with a 39% decrease. Shoot biomass was globally influenced by W management but not CC or N (Table 2). Nonetheless, shoot biomass decreased by 22% due to the presence of jack bean in the -W treatments and -N treatments (Fig. 7b). Finally, the root to shoot ratio was significantly influenced by the interaction between W and N management but not by CC management (Table 2, Fig. 7c).

Table 2. Statistical results of the analysis of variance on the variable root biomass, shoot biomass and root to shoot ratio

	Root biomass		Shoot biomass		Root to shoot	
	Df	p	Df	p	Df	p
±CC	1	0.0766	1	0.1246	1	0.9733
±W	1	0.0011**	1	0.0130*	1	0.0537
±N	1	0.1185	1	0.4632	1	0.0601
W x N	1	0.1230	1	0.0809	1	0.0207*
Other interactions		ns		ns		ns

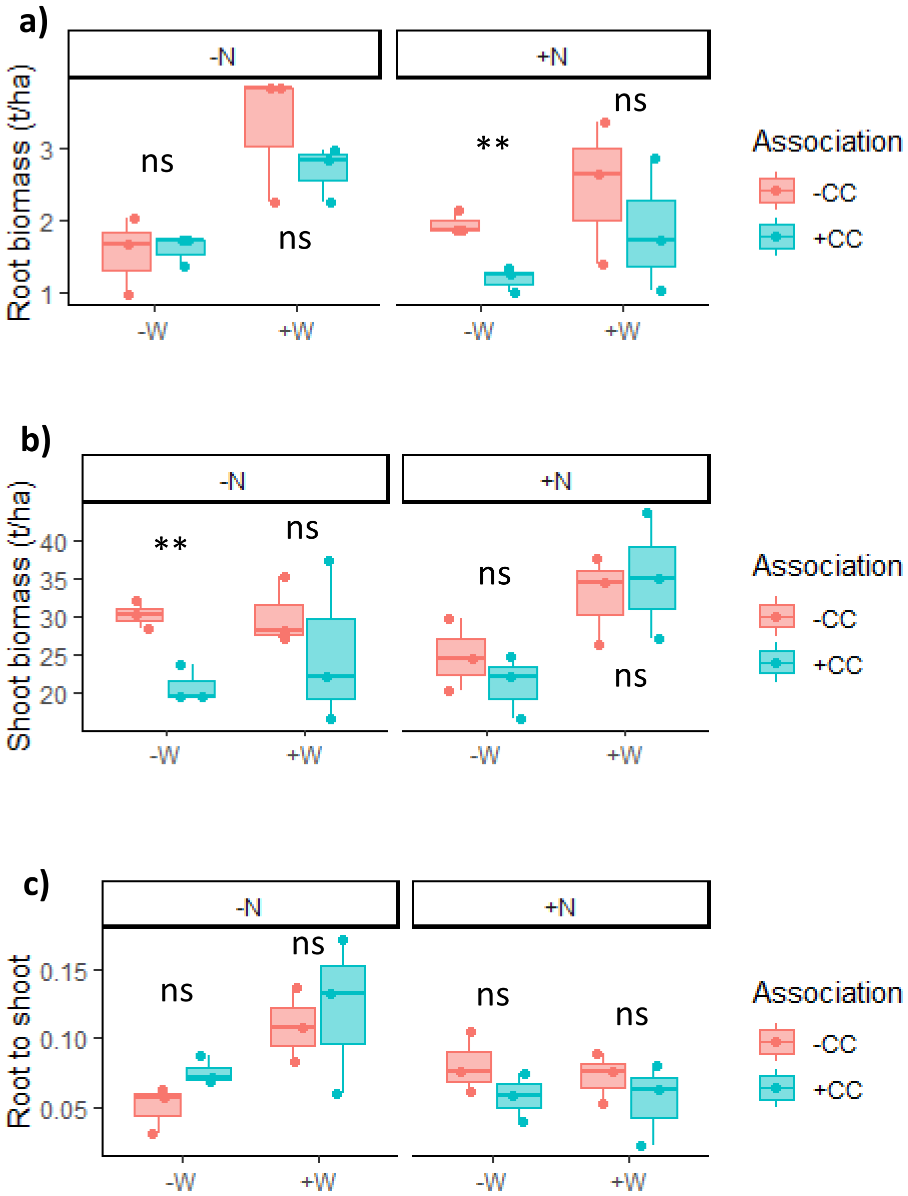

Fig. 7. Root biomass down to 50 cm (a), shoot biomass (b) and root to shoot (c) of sugarcane depending on companion crop treatment (CC), irrigation (W) and nitrogen (N) managements.

5.2. Impact of jack bean on sugarcane root density distribution

The cane root density was significantly influenced by the interaction between depth and position. It was also influenced by the presence or absence of jack bean (Table 3). Considering all treatments, the presence of jack bean seems to impact cane root density in the row (Position 1) rather than the interrow (Position 3, Fig. 8), even if there was no interaction effect between crop association and row position or depth. In the row, the root density of the cane tended to be lower in +CC than -CC in depths 0-10 cm and 10-30 cm. In the row, cane root density was decreased by 40% at depth 0-10 cm and 46% at depth 10-30 cm (Table 4). In each depth, the effect of CC was not significant due to limited repetitions (Tukey post hoc test). In the interrow, the negative impact of CC on cane root density only occurred in the +W-N treatment (Fig. 9). Overall, root density decreased with depth below 30 cm, whether in pure or in association, and it also decreased with distance from the cane row.

Table 3. Statistical results of the analysis of variance on the root density (g dm-3)

	Density	
	Df	p
±W±N	3	< 0.001
±CC	1	0.0412
Depth	1	< 0.0001
Position	2	< 0.0001
±W±N : ±CC	3	0.5327
±W±N : Depth	3	0.0018
±W±N : Position	6	0.0455
±CC : Depth	1	0.6730
±CC : Position	2	0.1984
Depth : Position	2	0.0026

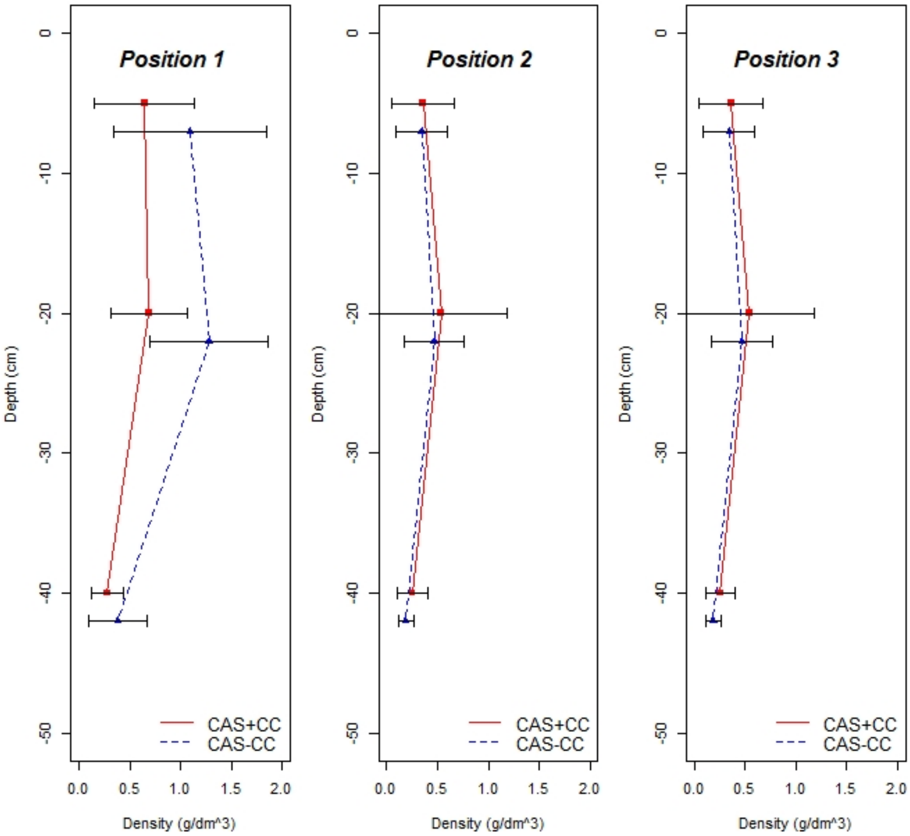

Fig. 8. Cane (CAS) root density depending on depth and CC treatment in the three distances from the row (position 1,2 and 3) With position 1 the closest to the row and position 3 the farthest to the row.

Table 4. Sugarcane (CAS) root density depending on depth and CC treatment in the three distances from the row (position 1,2 and 3).

	Depth (cm)		Density (g.dm^{-3})		Depth (cm)		Density (g.dm^{-3})		Depth (cm)		Density (g.dm^{-3})
Position 1	[0-10]	CAS	1.09 ± 0,76	Position 2	[0-10]	CAS	0.34 ± 0.25	Position 3	[0-10]	CAS	0.23 ± 0.27
	[10-30]		1.28 ± 0.58		[10-30]		0.47 ± 0.30		[10-30]		0.25 ± 0.25
	[30-50]		0.38 ± 0.29		[30-50]		0.19 ± 0.08		[30-50]		0,20 ± 0.15
	[0-10]	CAS+CC	0.65 ± 0.49		[0-10]	CAS+CC	0.36 ± 0.31		[0-10]	CAS+CC	0,15 ±0.13
	[10-30]		0.69 ± 0.37		[10-30]		0.54 ± 0.64		[10-30]		0,23 ± 0.19
	[30-50]		0.28 ± 0.16		[30-50]		0.25 ± 0.14		[30-50]		0,10 ± 0.06

5.3. Influence of nitrogen and water availability on sugarcane root

Sugarcane cane root biomass was significantly influenced by the ±W±N management and the interaction of depth and position (Table 3).

Total root biomass tended to be higher under -N than +N in the +W treatment (Fig. 7). In pure cane (-CC), root biomass was 3.29 t ha^{-1} and 2.45 t ha^{-1} in the +W-N and +W+N treatments, respectively. In association (+CC), root biomass was 2.67 t ha^{-1} and 1.85 t ha^{-1} in the +W-N and +W+N, respectively. These values corresponded to an increase by 25% in pure and 30% in association due to the absence of N fertilization. Additionally, root biomass was significantly higher in irrigated than in non-irrigated conditions. In average, root biomass increased by 47% with irrigation in the -N treatments and by 27% in the +N treatments.

Cane root density responded in a similar way than total root biomass under contrasted N and W managements (Fig. 9). 80% of the roots were found in the first 30 cm whether in pure cane or with CC. An exception was observed in the treatment with the highest root density (+W-N) where 90% of the roots were in the first 30 cm. Additionally, more than 50% of the cane roots were in position 1, i.e. the closest to the cane row (12.5 cm of the cane). It can be concluded that even if the values of density were different between N and W treatments, the overall trends of root vertical and horizontal distributions were the same.

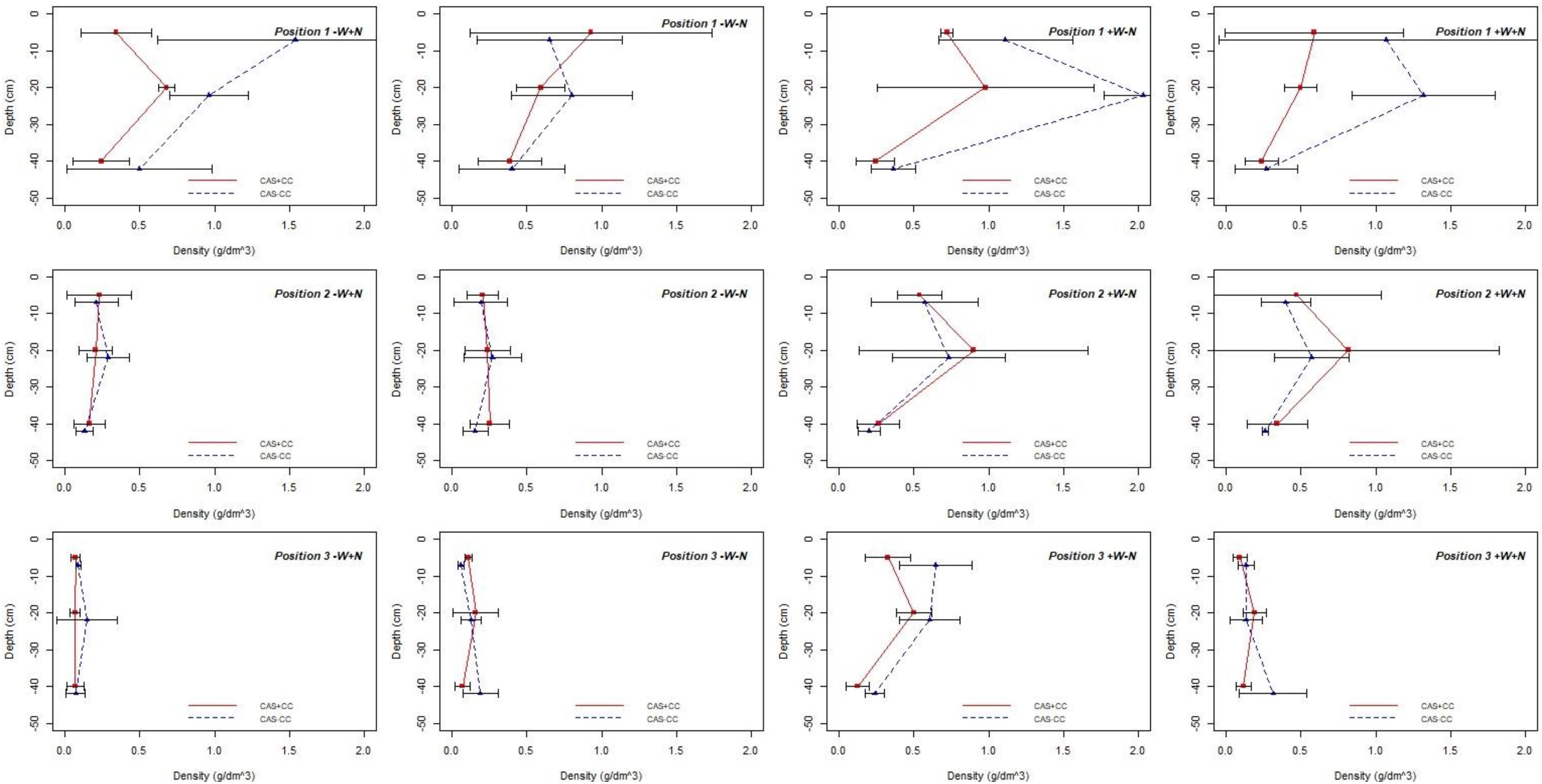

Fig. 9. Cane root density distribution depending on depth, position in the row and N, W and CC management.

5.4. Influence of nitrogen and water availability on jack bean root

Total root biomass of jack bean was influenced by N, W and the site location (Table 5). In the SD trial, Jack bean roots was higher by 43% under +N than -N (Fig. 10, Table 6). Jack bean root were also higher by 18% in -W than +W. In the SALSA trial the effect was more pronounced. In this trial, Jack bean roots was higher by 51% under +N than -N (Fig. 10, Table 6). Jack bean root were also higher by 40% in -W than +W.

Table 5. Statistical results of the analysis of variance on the variable root biomass (t ha-1)

	Root biomass (t ha^{-1})	
	Df	p
±W	1	0.0047
±N	1	0.0086
Site	1	1.74E^-08
Interaction	-	ns

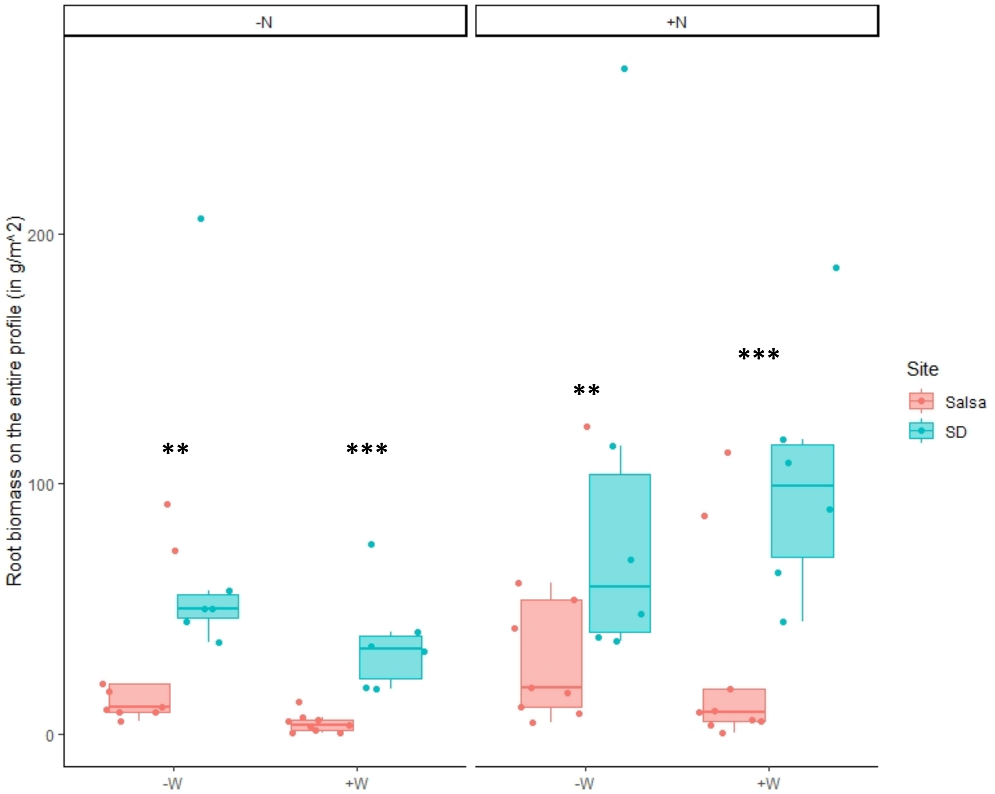

Fig. 10. Root biomass of jack bean according to the factor and the presence or absence of sugarcane

Table 6. Root biomass of jack bean according to the factor and the presence or absence of sugarcane

	Factor	Biomass (t ha^{-1})		Factor	Biomass (t ha^{-1})
Salsa	-W-N	27,11 ± 32.12	SD	-W-N	74,27 ± 65.12
	-W+N	37,46 ± 38.02		-W+N	95,76 ± 88.33
	+W-N	4,29 ± 3.86		+W-N	36,8 ± 21.3
	+W+N	27,73 ± 41.76		+W+N	101,1 ± 49.53

6. Discussion

6.1. Jack bean and sugarcane competition

A reduction in sugarcane root density was observed in the presence of jack bean in the cane row. This reduction could result from a competition between sugarcane and jack bean. To our knowledge, this study provides the first direct assessment of the influence of companion crops on sugarcane roots biomass. The impact on aerial dry mass was not always following the change in root biomass, suggesting a plasticity in root to shoot ratio depending on conditions.

Previous studies have highlighted competition effects between companion crops and sugarcane. As an example, a study carried out in Mexico on two companion crops in association with sugarcane have shown that *Canavalia ensiformis* caused decrease in aerial yield compared to the other companion crop: *Cajanus cajan*. The observed sugarcane yields were 62 t ha^{-1} for *Canavalia ensiformis* and 102 t ha^{-1} for *Cajanus cajan*, i.e. a 52% decrease in yield in the presence of *Canavalia ensiformis* (Córdova-Gamas et al., 2016). In addition, jack bean would have caused a 2-month delay in sugarcane growth. However, the study has also indicated that *Canavalia ensiformis* helped to control weeds more effectively, thus reducing herbicides use. In another study conducted this time in Reunion Island, *Canavalia ensiformis* was found to reduce aerial sugarcane yields by 18% (Mansuy et al., 2018). We can assume that these decreases in aerial yields are reflected in root density and that competition between jack bean and sugarcane leads to reductions in sugarcane root density.

More specifically, Ms. Pauline Viaud's thesis focuses on the evaluation of the capacity of multi-specific sugarcane/legume systems to improve resource use efficiency (water, nitrogen and light). Thus, one of the objectives of this thesis is to evaluate the competition processes between sugarcane and the companion crop, on water, nitrogen, and light resources. In this thesis the companion crop studied is the legume C*anavalia ensiformis*. As far as urea is concerned, preliminary results have shown that there was no competition between the two species (less than 5% of urea was used by the companion crop in ratoon crop) in the same trial that the one studied here (SALSA trial). Another study conducted on sugarcane and soybean in northeast China have shown the opposite results, observing competition for nitrogen resources (Yang et al., 2013). Considering water competition, previous studies performed in the SALSA trial have shown a decrease in water content by 10% due to jack bean companion crop in the -W treatments in the surface horizon (0-30 cm) (preliminary result of Pauline Viaud's thesis). In our study, jack bean produced more roots with 18% more root biomass in the -W treatments compared to the +W treatment. By opposition, the cane produced more roots in the +W condition. However, we were able to notice that for the -W+N modality, the cane in association with jack bean produced more roots on the whole profile far from the jack bean row compared to a cane without jack bean. As the cane in association produces more root at this position on the whole profile compared to a pure cane, we can hypothesize that the cane develops its root system preferentially in the position furthest from the jack bean in order to take up the resources necessary for its development. Such observation has been shown in various studies, notably in agroforestry systems where plants exploit root plasticity to avoid excessive root competition and to explore different regions of the soil (Schroth, 1998). However, as we were able to observe cane roots in the presence of jack bean, we hypothesise that these roots were in competition for water. Furthermore, as jack bean produced more roots under -W conditions in the Salsa trials, the pressure on the water resource is increased in the -W modalities, which have led to competition for water with cane. Another study has also shown that the introduction of legumes can led to competition for water with the main crop (Otto et al., 2020).

Some studies have shown that the introduction of cover crops in sugarcane interrow can led to competition for light or nutrients when the sugarcane starts growing again (Resende, et al., 2003). This was not the case here because the jack bean was sown 1.5 month after the sugarcane harvest. The cane has therefore had time to develop its roots to avoid competition for nutrients and it had time to grow in height, which limits competition for light. The opposite effect was even observed on the Salsa trial, with a 33% reduction in aboveground biomass of jack bean in the +W treatments, because the cane has grown faster and therefore decrease light availability for the companion crop. We hypothesize that the same competition process explained the lower root jack bean biomass observed in the +W condition on Salsa than on SD trial. However, the soils were also different between Salsa and SD trials which could also explained the difference in jack bean root biomass between the two trials.

The choice of the legume species, the size of the row and the ±N, ±W management are therefore essential to allow the complementarity and facilitation processes to be more important than the competition processes. This balance is very crucial for the success of a crop association (Duchene et al., 2017).

6.2. Cane root response to nitrogen and water availability

In the Salsa trial the influence of nitrogen availability on root biomass did not emerge significantly. As there was also little difference in yield under fertilized or unfertilized conditions for the above-ground biomass of cane, the hypothesis made was that the soil at Salsa was non-limited enough in nitrogen to impact cane growth (table 1). However, our results have shown that when looked at the effect of fertilisation under irrigated conditions the root density was higher under unfertilised conditions. A survey including 77 studies representing 206 cases and 129 species found that root density decreased with increasing N availability in the majority of the cases examined and would tend to underline the trend observed on Salsa (Reynolds, 1996). Another study carried out on sugarcane at two neighbouring sites of the Salsa trial have shown that nitrogen availability did not affect the lateral distribution of roots, but did affect their vertical distribution, with an increase in root density in the upper soil layers when fertilisation rates was reduced (Versini et al., 2020).

In contrast, another study conducted in 2011 have shown the opposite trend (Otto et al., 2011). The hypothesis they made was that roots concentrated on the surface in fertilised conditions (where fertiliser is supplied), while in unfertilised conditions they moved to the lower horizons to meet the demand of the cane. These contradictory results underline the importance of considering other pedoclimatic factors such as water availability. Indeed, we have noted that under unfertilized and irrigated conditions(+W-N), root density was higher in the 10-30 cm horizon and in the 0-10 cm horizon than in the 30-50 cm horizon. The assumption we made is the same as that made by Versini et al., (2020), that cane roots in unfertilized conditions will concentrate in the upper horizons where the greatest source of plant available nitrogen is found. A study conducted on maize have shown that under irrigated conditions, most of the available nitrogen for the crop was found at the surface (0-30 cm) and tends to confirm this hypothesis (Stanford, 1982).

Our results showed a contrasted distribution of roots between surface and depth, as well as according to the distance from the row. The observations we made with an average of 80% of the root density in the first 30 cm and more than 50% of the root density close to the row were in line with the observations made in a literature review. Similarly, Blackburn et al. (1984) showed that 50% of the sugarcane roots were found in the first 20 cm of soil. The study by Versini et al., (2020) carried out in La Réunion also showed that for a 6-month-old cane, 70% of the roots were found on average in the first 30 cm of the soil. This study also confirms that more than 50% of the root density was found near the row.

6.3. Separation of the root niches

Previous results on cane roots in plant crop (eRcane, 2014) as well as in the previous ratoon (2019-2020) in the same trial showed a decreasing trend in cane root density in regrowth in the presence of jack bean in the interrow. For the year 2020-2021, it was possible to observe the same trend close to the cane row with slight variation for some modalities (-W-N). However, the niche separation seemed much less important than in the previous year. The hypothesis made is that the cane having more time to develop its roots outside the jack bean cycle, will be able to colonise the inter-row. Thus, the following year when the jack bean is sown the cane roots are already present in the row and the niche separation disappears during the cane cycles. Although, even if the research is scarce on the impact of companion crop roots on sugarcane roots, a 3-year study in Santa-Fe, Brazil, showed similar results. Indeed, they note that the companion crop influenced root development only during the first two cycles of the crop, when the cane has not completely colonised its inter-row (Lovera, 2021).

Later, by analysing the additional data collected through the circle method, it will be possible to assess more precisely the impact of the jack bean roots on the cane roots directly under the jack bean row and thus confirm or refute the hypothesis of a root niche separation between the two species. In view of the literature and what we observe, we expect to find no niche separation in this second ratoon crop.

7. Conclusion and perspectives

The root response of sugarcane in the presence of companion crops and under contrasting conditions of nitrogen and water availability can be described as follows: i) The presence of companion crop seem to impact the sugarcane root density in the row rather than in the interrow, ii) cane root density decreased with depth and distance from the cane row regardless of the modality studied. 80% of the root density was found in the first 30 cm of soil and more than 50% of the root density was found closest to the cane row, whether in pure or in association, iii) a greater total cane root biomass and root density down to 30 cm was observed in +W condition with an increase of 47% in -N and 27% in +N. The opposite trend was observed for jack bean roots. Jack bean root biomass increased by 18% under water stress. As no difference were found in deep soil layers, water availability would thus influence sugarcane roots essentially in the upper soil layers.

In view of the preliminary results, we did not seem to observe any niche separation between sugarcane roots and jack bean roots. The study of the data produced with the circle method will allow us to confirm or refute this hypothesis. In addition, the integration of data on root length and size will allow us to know more finely the root distribution according to thin or coarse roots. Knowing where these different types of roots are distributed will give us an additional indication of the separation or not of root niches between jack bean and sugarcane. Indeed, fine roots are the seat of absorption for nutrients and water. This knowledge will allow us to better understand the processes underlying the competition between the two species.

The data produced will later be used for two PhD theses. Firstly, this data collection is necessary for a part of a thesis on modelling the sugarcane-legume association using the STICS model. The data produced in this study will be used to model the root compartment of the sugarcane and the companion crop separately. Finally, the data will be used to write a scientific article for part of my thesis on the effects of reducing synthetic inputs on the root development of sugarcane in Reunion Island. The question that we will try to answer in relation to these data is: how does the reduction of herbicides and the maintenance of grass cover (spontaneous or introduced) in the cane inter-row impact the growth dynamics, the distribution of cane roots and the access to resources?

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