How the use of nitrogen fertiliser may switch plant suitability for aphids: the case of Miscanthus, a promising biomass crop, and the aphid pest Rhopalosiphum maidis

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How the use of nitrogen fertiliser may switch plant suitability for aphids: the case of Miscanthus, a promising biomass crop and the aphid pest *Rhopalosiphum maidis*.

Running title: Nitrogen fertilizer and Miscanthus-aphid complex

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Abstract

BACKGROUND: The use of nitrogen fertiliser in agrosystems can alter plant nitrogen and consequently improve nutrient availability for herbivores, potentially leading to better performance for herbivores and higher pest pressure in the field.

RESULTS: We compared, in laboratory conditions, the effects of nitrogen fertilisation on a promising biomass crop, *Miscanthus* x *giganteus*, and its parents *Miscanthus sinensis* and *Miscanthus sacchariflorus*. The plant-mediated effects were compared on the second trophic level, the green corn leaf aphid *Rhopalosiphum maidis*.

Results showed that the biomass and leaf C:N ratio of *M. sinensis* plants treated with nitrogen fertiliser were significantly greater than those of non-treated plants. Concerning *M.* x *giganteus* and *M. sacchariflorus*, the only reported change was a significantly smaller leaf C:N ratio for treated *M. sacchariflorus* compared to non-treated plants.

Surprisingly, nitrogen fertilisation had opposite consequences on plant-herbivores interactions. Following N treatments, *M. sinensis* was less suitable in terms of intrinsic rate of increase for *R. maidis*, whose feeding behavior was negatively affected, while *M. sacchariflorus* and *M.* x *giganteus* exhibited greater suitability in terms of aphid weight.

CONCLUSION: Nitrogen fertilisation had contrasting effects on the three species of Miscanthus plants. These effects cascaded up to the second trophic level, *R. maidis* aphid pests, either through a modification of their weight or demographic parameters. The implications of these results were discussed in the context of agricultural sustainability and intensive production practices.

Keywords: Aphididae, Aphid performance, Electropenetrography, Leaf C:N ratio, Miscanthus species, Nitrogen input, Pest management

1. Introduction

Nitrogen (N) is a key element for plants. Its availability in agrosystems can be improved with the use of fertilisers. Plants are capable of plastic responses following fertilising, as evidenced by the profound reprogramming of their N and carbon (C) metabolism.[1] Such metabolic changes can impact plant quality and ultimately influence trophic-level interactions, thus affecting the performance of herbivores.[2] Plant response following the use of fertiliser may vary between different species but also between cultivars as shown by various studies on maize or oilseed rape.[3,4] Nitrogen inputs have usually been linked with decreased C:N ratio in plants, correlated to an improvement of plant quality for herbivores.[5] A higher plant N content has been shown to positively impact their feeding behaviour[6] or life history traits[5,7,8], and thus pest pressure in the field. However, excessive nutrient intake by herbivores can negatively affect their fitness and population dynamics.[9] Altogether the N content may influence plant resistance to higher trophic levels, mostly through the alteration of primary and secondary metabolites production in plants.[1,10] According to the Carbon-Nutrient-Balance hypothesis,[11] a plant subjected to an abundant amount of nitrogen should allocate relatively more to nitrogen-containing defence metabolites and reduce secondary carbon-based substances.

Nitrogen requirement is a particularly significant issue, since both the manufacturing process of nitrogen fertilisers and losses following application can have local and global environmental impacts as well as significant implications to greenhouse-gas balances.[12] Smith *et al*.[13] reported that biomass crops have great potential to mitigate carbon emissions and are likely to be major contributors to the renewable energy mix in the future. *Miscanthus* x *giganteus,* a vigorous sterile hybrid between *M. sinensis* and *M. sacchariflorus,* is a promising crop dedicated to biomass and biofuel production. The potential of Miscanthus is attributed to high productivity and long term perenniality, together with nutrient requirements that are generally considered low, although the exact needs of this crop are not yet defined.[14]

The two cultivated species of Miscanthus exhibit different productivities, the crops of the sterile hybrid *M.* x *giganteus* leading to higher yields than those of the parental species *M. sinensis*. However, as European crops of *M.* x *giganteus* consist of a single clone, *M. sinensis* is still regarded as a potential alternative for Miscanthus production as certain *M. sinensis* clones display a high biomass potential.[15,16] Although the parental species *M. sacchariflorus* is not a dedicated biomass crop, it is also interesting as a progenitor for breeding programs, due to its low ash content making it suitable for the different bioenergy conversion processes.[17] Interestingly, the three species of Miscanthus differ in terms of susceptibility to pests, in particular to aphids.[18] The corn leaf aphid *Rhopalosiphum maidis* is considered as the main Miscanthus pest, as *R. maidis* colonies can develop on Miscanthus host-plants[19], to which they can also transmit the *Barley yellow dwarf virus*[20]. Indeed, some studies using vitro-plants have pointed out that *M. sacchariflorus* shows a greater resistance to the corn leaf aphid *R. maidis* than *M. sinensis* and *M.* x *giganteus*.[21,22] Another study using potted plants has shown that *M. sinensis* was more resistant than *M.* x *giganteus*, raising the possibility that *M. sinensis* could represent a better alternative to *M.* x *giganteus* under heavy aphid pressure.[20] These studies suggest that Miscanthus breeding programs should also take into account traits that are related to resistance to insect pests. [21]

To understand the possible consequences of fertilisers on pest pressure in bioenergy crops, we compared the effects of nitrogen input on the interactions between three species of Miscanthus (*M.* x *giganteus*, *M. sinensis* and *M. sacchariflorus*) and the green corn leaf aphid *Rhopalosiphum maidis*. To date, regarding the different nitrogen supply programs studied, none has converged towards a consensual understanding of Miscanthus response to fertilisation. This might be attributed to the fact that, so far, the majority of these studies were solely performed in field setups and therefore submitted to variations and potential biases inherent to natural conditions.[12] The aim of the present study was to compare, in controlled and standardised conditions, the effects of nitrogen input on *M.* x *giganteus* and its two parents, as well as the plant-mediated effects on the second trophic level, the aphid *R. maidis*. We predicted that nitrogen inputs should positively impact (i) the plants biomass and N contents; (ii) the feeding behaviour of aphids; (iii) the performance of aphids reared on these host-plants, including weight and demographic parameters.

2. Materials and Methods

2.1 Insects and Plants

2.1.1 Insect cultures

A laboratory colony of the aphid *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) was initiated from a parthenogenetic aphid population. Aphids were reared on plants of winter barley (*Hordeum vulgare* cv. "Cervoise"). Pots (90 × 90 × 70 mm) containing each 15–20 barley plants were placed in ventilated Plastic ® cages (240 × 110 × 360 mm) and maintained in a growth chamber under 20 ± 1°C, 60 ± 5 % RH, and a 16:8 L:D light cycle.

2.1.2 Plants material

Plantlets of the three Miscanthus species, *i.e.*, *M.* x *giganteus* (cv. "GigB", 2n = 3x = 57), *M. sacchariflorus* (cv. "Sac", 2n = 2x = 38) and *M. sinensis* (cv. "Goliath", 2n = 4x = 76)[23] were obtained by *in vitro* multiplication as described in previous study.[24] Single rooted shoots coming from clusters, rooted in perlite for eight weeks, were transplanted into plastic pots (firstly 9 × 9 × 10 cm, then 13 × 16 cm and 15 × 20 cm (height x diameter)) containing potting soil (NPK 18-10-20, 0.5 kg/m^3, FLORAGARD) and kept in a growth chamber under 20 ± 1°C, 60 ± 5 % Relative Humidity (RH), and a 16:8 (L:D) photoperiod for ten weeks.

2.2 Nitrogen treatments

After development for ten weeks in the growth chamber, the potted plants were randomly assigned to one of the two following treatments: (i) a low nitrogen input, consisting of the potting soil only (non-treated, referred to as "-N") (ii) potting soil supplemented with 0.76

gN.plants^{-1}of ammonium nitrate (treated, referred to as “+N”). This dose of nitrogen (*i.e.* 120 kgN.ha^{-1}) was chosen in accordance with previous works.[25] Application of the nitrogenous solution was carried out using two half-doses. The first half-dose (0.38 gNH_4NO_3) was diluted in 100 mL of water and was applied over the entire soil surface of each pot three weeks before use for the experiments. The second half-dose was applied one week after the first one. All Miscanthus plants were placed randomly (*i.e.* regardless of the species or nitrogen status) in a greenhouse under controlled conditions (20 ± 1 °C, 60 ± 5 % RH, photophase 16h at 4 klux) for three weeks after the first N application, until they were used for experiments (plant biomass measurement and introduction of aphids onto the plants), *i.e.* thirteen weeks following potting. Watering was performed every 48 hours using volumes (100 ml) of water that had been calibrated during preliminary tests to prevent any leachage. No experimental blocking was performed.

2.3 Effects of nitrogen treatments on plants aerial biomass and quality

2.3.1 Plants biomass measurements

For each treatment and species, plant sprouts were individualised and weighed using an electronic balance (Mettler Toledo ML204, Max: 220 g, d = 0.1 mg), then placed in the freezer at -80 °C for a later use in carbon and nitrogen content measurements as described in previous works.[26] For *M. sacchariflorus*, twelve plants were weighed for the "-N" treatment and seven for the "+N" one. Eleven plants of *M.* x *giganteus* were weighed for the "-N" treatment and ten plants for the "+N" one. For *M. sinensis*, ten plants were weighed for each treatment ("-N" and "+N").

2.3.2 Plants quality

The quality of plants was assessed through the calculation of the Carbon:Nitrogen (C:N) ratio of their aerial organs. To perform the measurement of the C and N contents, the samples from the previous experiments (stored at -80 °C) were lyophilised at 4 °C for 48 hours using a lyophiliser (Alpha I-5). Samples of Miscanthus plantlets were processed individually. Each sample was ground for one minute with a ball mill (Retsch MM 400), then placed in a tin basket and weighed using a precision balance (Sartorius Genius ME415S). The carbon and nitrogen concentrations were determined using an elemental analyser (Flash EA 1112 series Thermo Electron, Bremen Germany). For *M. sacchariflorus*, twelve plants were analysed for the "-N" treatment and ten for the "+N" one. Eleven plants of *M.* x *giganteus* were analysed for each treatment ("-N" and "+N"). For *M. sinensis*, ten plants were analysed for each treatment ("-N" and "+N").

2.4 Plant-mediated nitrogen effects on *R. maidis* feeding behaviour

The electrical penetration graph DC-system was used.[27] To insert one aphid and one plant into an electrical circuit, a thin gold wire (20 mm diameter and 2 cm long) was tethered on the insect's dorsum using conductive silver glue (EPG systems, Wageningen, The Netherlands). Eight aphids were connected to the Giga-8 DC-EPG amplifier and each one was placed on the leaf of an individual plant. A second electrode was inserted into the soil of each of the potted plants to complete the electrical circuits. The recordings were performed continuously for six hours during the photophase. Each aphid-plant system was placed as a whole inside a Faraday cage at 20 ± 1 °C. Acquisition and analysis of the EPG waveforms were carried out with PROBE 3.5 software (EPG Systems, www.epgsystems.eu). Parameters from the recorded EPG waveforms were calculated with EPG-Calc 6.1 software.[28] These parameters were based on different EPG waveforms corresponding to:[29] (C) stylet pathways in plant tissues except phloem and xylem; (pd) potential drops (intracellular stylet punctures); (E1) salivation in phloem elements; (E2) passive phloem sap ingestion; (E1 + E2) activity within phloem vessels, (G) active xylem sap ingestion; and (F) derailed stylet mechanics. In this study, the feeding behaviour of *R. maidis* on *M. sinensis*, *M. sacchariflorus* or *M. x giganteus*, treated or not, was investigated using 24–39 individuals.

2.5 Plant-mediated nitrogen effects on *R. maidis* aphid performance

Pools of synchronised first instar nymphs (less than 24-hours old) were obtained from parthenogenetic adult females placed on leaves of their host-plant set in 1.5 % agar in Petri dishes (90 mm diameter). To obtain synchronised young adults, first instar nymphs were kept in the same device for a further eight days. For the nymph survival study, groups of five first instar nymphs were gently transferred, using a small paintbrush, onto the plantlets to be tested. These groups of aphid nymphs were enclosed in plastic clip-cages (15 mm diameter, 10 mm height) ventilated by a grid and held by a metal clip on the lower face of a leaf at the mid-height of each plantlet, and their survival was recorded every day. To study the adult performance, young adults were individually transferred onto the plantlets to be tested. Survival and fecundity of adult individuals were recorded every 24 hours. Each adult individual was placed individually in a clip-cage in order to count the number of nymphs produced. The newly larviposited individuals were counted and removed with a brush every 24 hours to estimate the daily fecundity of each individual parent. A total of 120 adult aphids were followed, with 20 aphids for each host-plant species and each treatment. Their daily fecundity was assessed for a duration equivalent to twice the pre-reproductive period duration. The intrinsic rate of natural increase (r_m) was calculated using the DEMP 1.5.2

Software,[30] as $\Sigma e^{-r_m x} l_x m_x = 1$, where x was the age, l_x the age-specific survival, and m_x the mean number of female offspring produced in a unit of time by a female aged x.[31] This parameter was selected to compare the ability of *R. maidis* to establish a population on each of the three Miscanthus species treated or not with NH_4NO_3 addition.

To measure aphid weight, synchronisations of aphids were carried out in plastic boxes (125 x 115 x 55 mm) on each of the three species of Miscanthus, for the two treatments ("-N" and "+N"). A 30 cm^2 opening covered with nylon mesh was made on each box for ventilation. A single Miscanthus leaf was slid inside each box, on which 100 newly larviposited *R. maidis* nymphs were deposited. For each species of Miscanthus, eight plants were used for the experiments, with four plants per treatment ("-N" and "+N"). For each plant, four plastic boxes containing a leaf with 100 aphids were used. At eight days post-larviposition, 30 aphids were randomly selected from each box and individually weighed using an electronic precision balance (Mettler M3, class 1, Max: 3g Low: 1 µg, T = -3G [dd] = 1 µg).

2.6 Statistical analyses

As a Shapiro-Wilk test showed that data were not normally distributed, non-parametric tests were used for all analyses. The impacts of nitrogen fertilisation within the three species of Miscanthus on the aboveground biomass, leaf nitrogen content and leaf C:N ratio were analysed using Mann–Whitney *U* tests. Plant-mediated nitrogen effects on the feeding behaviour of *R. maidis* were analysed using Mann–Whitney *U* tests. The combined effects of the Miscanthus host species and N treatment on *R. maidis* performance parameters and weight were analysed using GLM with quasipoisson distribution (link: log) followed by pairwise comparisons using least-squares means (package R: "lsmeans"). All statistical analyses were performed in the R software.[32]

3. Results

3.1 Effects of nitrogen addition on aboveground biomass and quality of the three species of Miscanthus

Following addition of NH_4NO_3 (+N) on *M. sinensis*, the biomass of plants was significantly greater than that of non-treated (-N) *M. sinensis* plants (U = 19, P = 0.007) (Table 1a). For *M.* x *giganteus* and *M. sacchariflorus*, the addition of NH_4NO_3 had no significant effect on the aboveground biomass of plants (*M.* x *giganteus*: U = 45, *P* = 0.332; *M. sacchariflorus*: U = 66, *P* = 0.722) (Table 1b and 1c). The C:N leaf ratio of *M. sinensis* exposed to NH_4NO_3 (+N) was significantly greater than that of non-treated (-N) *M. sinensis* plants (U = 19, P = 0.007) (Table 1a). For *M.* x *giganteus*, the leaf C:N ratio was not significantly affected by the treatment (U = 65, *P* = 0.797) (Table 1b). The leaf C:N ratio of treated (+N) *M. sacchariflorus* was significantly smaller than the leaf C:N ratio of non-treated (-N) *M. sacchariflorus* plants (U = 93, *P* = 0.035) (Table 1c). NH_4NO_3 treatment did not have any significant effect on leaf nitrogen content for *M.* x *giganteus* (U = 59, *P* = 0.949) (Table 1b and 1c). Treated (+N) *M. sinensis* and *M. sacchariflorus* respectively showed significantly lower and higher leaf nitrogen content compared to non-treated (-N) plants (*M. sinensis:* U = 93, *P* = 0.021; *M. sacchariflorus*: U = 24, *P* = 0.017) (Table 1a).

3.2 Plant-mediated nitrogen effects on aphid feeding behaviour

Whatever the modality (plant species or nitrogen treatment), individuals exhibited sustained phloem sap ingestion (E2). During the six hours of recording, the total duration of stylet activity in the plant lasted on average about five hours. When submitted to treated (+N) *M. sinensis* plants, *R. maidis* aphids exhibited a significantly longer duration of the pathway phase (C) (U = 370, P = 0.003) and a significantly shorter hydration phase (G) (U = 839, P = 0.0004) than aphids on non-treated (-N) *M. sinensis* plants. On treated (+N) *M. sacchariflorus* plants, aphids derailment phase of stylets (F) was significantly shorter than on non-treated (-N) *M. sacchariflorus* plants (U = 399, P = 0.024). On treated (+N) *M.* x *giganteus* plants, *R. maidis* salivation period (E1) was significantly shorter than on non-treated (-N) *M.* x *giganteus* plants (U = 261, P = 0.037) (Table 2).

3.3.Plant-mediated nitrogen effects on aphid performance

The average rate of natural increase r_m of *R. maidis* aphids was significantly affected by the plant species (χ^2 = 0.676, df = 127, $P = 1.238 \times 10^{-14}$) and by the N treatment (χ^2 = 0.044, df = 126, P = 0.041). The r_m was significantly lower when aphids were reared on *M.* x *giganteus* compared to *M. sinensis* or *M. sacchariflorus*. When aphids developed on treated (+N) *M. sinensis*, the r_m was significantly smaller compared to that of aphids on non-treated (-N) *M. sinensis* plants. There was no interaction between the two factors (χ^2 = 0.026, df = 124, P = 0.286) (Fig. 1a.).

The weight of *R. maidis* aphids was significantly affected by the plant species (χ^2 = 1231.87, df = 177, $P = 2.848 \times 10^{-14}$) and by the N treatment (χ^2 = 2131.62, df = 176, $P < 2.2 \times 10^{-16}$) (Fig. 1b.). There was a significant interaction between the plant species and the N treatment (χ^2 = 932.49, df = 174, $P = 5.579 \times 10^{-11}$) (Fig. 1b.). The weights of aphids developing on non-treated (-N) *M.* x *giganteus* were significantly smaller than those on non-treated (-N) *M. sinensis*. The weights of aphids developing on treated (+N) plants were significantly greater on *M.* x *giganteus* (2.5 times) and *M. sacchariflorus* (4 times) compared to those developing on the respective non-treated (-N) host-plants.

4. Discussion

To our knowledge, this is the first study not only reporting the effects of nitrogen input on the three main species of the Miscanthus genus, but also investigating the consequences on aphid herbivores. Nitrogen fertilisation had contrasting effects on the three species of Miscanthus plants. These effects cascaded up to the second trophic level, *R. maidis* aphids, either through a modification of their weight or demographic parameters.

We showed that nitrogen input had no significant impact on the aboveground biomass of the hybrid *M.* x *giganteus* and of the parental species *M. sacchariflorus*. To our knowledge, this is the first study of nitrogen input consequences on *M. sacchariflorus*. Our results are in line with those obtained in field conditions,[33,12] according to which nitrogen fertilisation had no impact on the aboveground biomass of *M.* x *giganteus* crops during the first years after rhizome transplanting. On the contrary, the aboveground biomass of the second parental species, *M. sinensis*, was significantly greater for treated (+N) plants.

In our study, nitrogen input differently impacted the C:N ratio of each Miscanthus species: *M.* x *giganteus* hybrid leaf C:N ratio was not affected by nitrogen input contrary to those of parental species. The leaf C:N ratio of treated (+N) plants was significantly smaller for *M. sacchariflorus* but significantly greater for *M. sinensis*. The results obtained on *M. sinensis* could be explained by the nitrogen dilution when the biomass increased.[34] Most studies show that decreased plant C:N ratios are generally linked to an increased biomass following nitrogen fertlisation.[25] The differences observed between the C:N ratio of the three species of Miscanthus could be due to their different use of nitrogen in our experimental conditions.

Previously published papers highlight the relationships between a high nitrogen content in the host-plant and a high performance in terms of population growth rate,[5] weight increase,[7] or both for the phytophagous.[8] Regarding the nitrogen leaf content of treated (+N) Miscanthus, we could have expected the performance (in terms of r_m and/or weight) of the *R. maidis* aphid to be (i) worse on *M. sinensis*, (ii) better on *M. sacchariflorus* and (iii) not different on *M.* x *giganteus* in comparison with the non-treated (-N) respective host-plants.

Consistent with our assumption about *M. sinensis*, aphids r_m was significantly smaller on treated (+N) plants (although their weight was not different), whose leaf C:N ratio was significantly higher. This seemed consistent with the study,[35] where a decrease of aphid performance was imputed to a high C:N in plant tissues. The significantly smaller *R. maidis* r_m measured on treated (+N) plants can be explained by the negatively altered feeding behaviour revealed by a significantly longer duration of stylet pathways (C) and shorter duration of xylem (G) phases. Ameline *et al*.[36] showed that an increase of this hydration phase induced better aphid demographic performance. Therefore, the alteration of *R. maidis* performance could be due to a decrease of xylem sap ingestion. However, the phloem sap intake duration was unchanged although phloem quality had possibly been lower, as indicated by the greater leaf C:N ratio. This could explain why *R. maidis* weight was not impacted and also, why its r_m was negatively affected.

Consistent with our assumption about *M. sacchariflorus*, *R. maidis* aphids weight was significantly greater in treated (+N) plants and their feeding behaviour seemed to be positively affected. Indeed, we recorded a significantly shorter duration of stylet derailment (F), a phase generally considered as an indicator of plant resistance.[26] The phloem sap intake duration was similar for both treatments although phloem quality was probably better in treated plants, as indicated by their smaller leaf C:N ratio. This could explain why *R. maidis* weight was so remarkably greater. However, despite this smaller leaf C:N ratio, aphid r_m was similar to that on non-treated plants.

On the treated (+N) hybrid *M.* x *giganteus*, aphids r_m was also similar to that on non-treated plants but their weight was greater. Nitrogen treatment did not affect the *M.* x *giganteus* leaf C:N ratio, contrary to both treated (+N) parents in which opposite effects were recorded (smaller C:N for *M. sacchariflorus* and greater C:N for *M. sinensis*). This could be the consequence of the hybrid status of *M.* x *giganteus*. Accordingly, the consequences of fertiliser use on *M.* x *giganteus* were less important on aphids than those on its parents. The changes of aphid feeding behaviour following N fertilising were minor.

The present study shows that aphids r_m and weight can be differently affected by the quality of their host-plants, as described in the theoretical model.[37] Indeed, according to this model, when the nutritional quality of the host is low, the herbivore will allocate or invest a greater amount of energy to its body weight than to the production of offspring, thus suggesting the existence of trade-offs. Indeed, in our study, the possible trade-offs between the parameters taken into account for r_m calculation and aphid growth depended on the host-plant: (1) on treated *M.* x *giganteus* and *M. sacchariflorus*, the host-plant quality was better, aphids weights were greater but their r_m unchanged; (2) on treated *M. sinensis*, the host-plant quality was lower, aphid r_m was smaller but the weight was unchanged.

In our study, we showed that nitrogen treatment could influence Miscanthus suitability for *R. maidis* aphids. When host-plants were not treated (-N), *R. maidis* aphids reared on the hybrid *M.* x *giganteus* showed smaller larval weights compared to those developing on *M. sinensis*, and smaller r_m compared to aphids on both parental species. These results appeared similar to the previous study,[18] demonstrating that *M.* x *giganteus* exhibited a stronger resistance to *R. padi* than *M. sinensis*, and that *M. sacchariflorus* was the most sensitive host-plant. Hence, both parents exhibited the best suitability for *R. maidis* regarding aphid weight and r_m. Following nitrogen input, *M. sacchariflorus* remained the most suitable host-plant. The other parent, *M. sinensis* was less suitable for *R. maidis* compared to (i) *M. sacchariflorus* when considering aphids r_m, and (ii) both *M. sacchariflorus* and the cultivated hybrid *M.* x *giganteus* regarding aphid weight. This switch in suitability could be attributed to the difference in C:N ratio between *M.* x *giganteus* and *M. sinensis*, and thus in their nutritional quality for aphids in terms of primary and/or secondary metabolites.[1,10] Other explanatory factors, such as physical features, can be ruled out considering the absence of significant interspecific differences between non-treated (-N) and treated (N) host-plants in the following EPG parameters: time to first probe, time to first phloem phase, duration of pathways phase (data not shown).

Most studies have shown that nitrogen fertilisation improves crop yields and also leads to enhanced pest pressure[2,8]. However, the results of our study showed this was not the case for the cultivated species of Miscanthus. Indeed, in the case of *M.* x *giganteus* and in line with the sustainable agriculture practices, we confirmed the previous fields results[12] as nitrogen fertilisation did not impact plant productivity in terms of aboveground biomass. Our work also suggested that the suitability of *M.* x *giganteus* for *R. maidis* pests was not affected by nitrogen fertiliser. In the case of *M. sinensis* and in line with the intensive production practices, nitrogen fertilisation did improve plant productivity, but *M. sinensis* suitability for pests was unexpectedly lower.

Finally, as Miscanthus crops can act as host reservoirs for the *Barley yellow dwarf virus,*[20,38] our results underline the importance to consider both the selected host-plant species and the agricultural practices in terms of fertility programs as they can modulate the population dynamics of aphids vectors.

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Tables

Table 1 Influence of nitrogen fertiliser treatment on biomass and quality of *M. sinensis* (a), *M. sacchariflorus* (b) and *M.* x *giganteus* (c) host-plants.

a)	*M. sinensis*		
Parameter	- N°	+ N†	Statistics‡
Aboveground biomass (g)	26.66 ± 3.23	48.85 ± 8.41	**
Leaf C:N ratio	14.71 ± 0.65	17.35 ± 0.44	**
Leaf nitrogen content (%)	3.23 ± 0.15	2.78 ± 0.06	*

b)	*M. sacchariflorus*		
Parameter	- N°	+ N†	Statistics‡
Aboveground biomass (g)	2.32 ± 0.44	2.16 ± 0.52	**NS**
Leaf C:N ratio	18.72 ± 1.55	15.43 ± 0.91	*
Leaf nitrogen content (%)	2.46 ± 0.17	2.95 ± 0.12	*

c)	*M.* x *giganteus*		
Parameter	- N°	+ N†	Statistics‡
Aboveground biomass (g)	23.95 ± 5.77	30.55 ± 6.65	**NS**
Leaf C:N ratio	15.60 ± 0.59	15.25 ± 0.36	**NS**
Leaf nitrogen content (%)	3.14 ± 0.12	3.19 ± 0.05	**NS**

Asterisks indicate statistically significant differences (Mann–Whitney *U tests, * P<0.05; ** P<0.01, ***: P<0.001)*

° Mean values ± SEM for plants without N solution (-N)

† Mean values ± SEM for plants with N solution (120 kgN/ha) (+N)

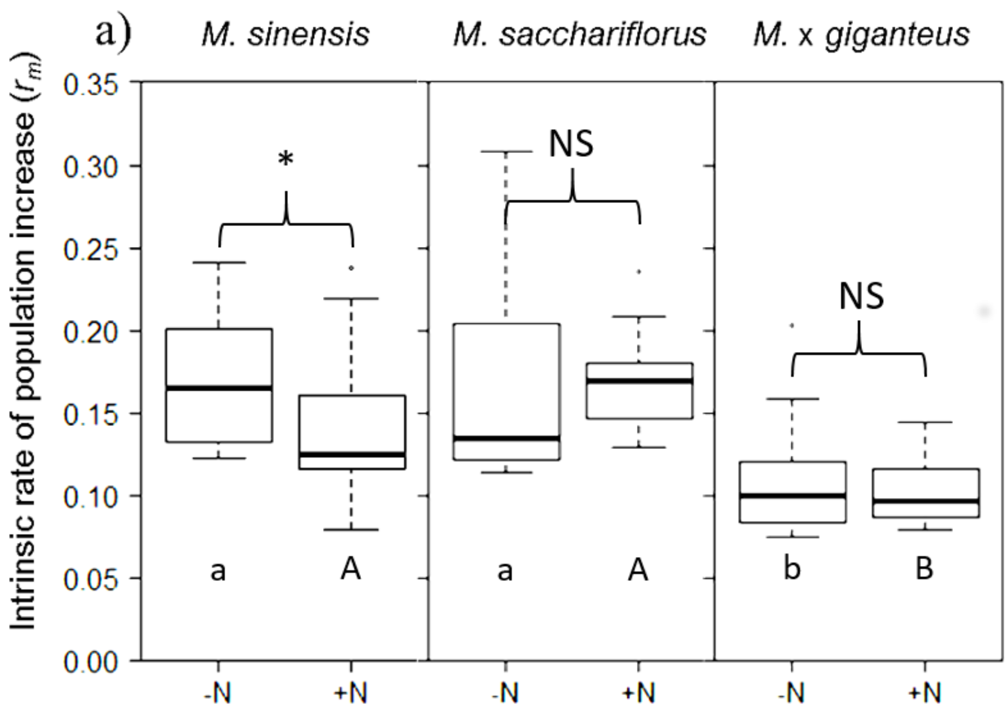
Table 2: Feeding behaviour parameters (mean ± standard error of the mean) of *R. maidis*, developing on *M. sinensis*, *M.* x *giganteus* or *M. sacchariflorus* host-plants on depleted soil (-N) or treated with a nitrogenous fertiliser 120 kg.N.ha^{-1} (+N).

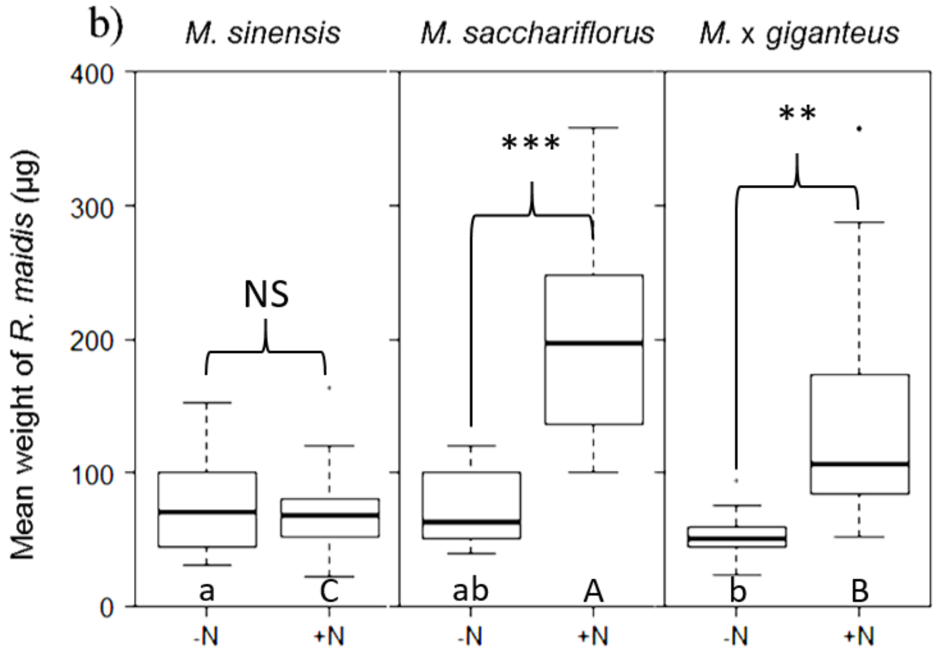
		M. sinensis (-N)	*M. sinensis* (+N)		*M.* x *giganteus* (-N)	*M.* x *giganteus* (+N)		*M. sacchariflorus* (-N)	*M. sacchariflorus* (+N)	
Parameter		n=32	n=39		n=38	n=24		n=37	n=38	
Pr	s_Pr	305.44 ± 9.86	292.69 ± 10.16	NS	308.04 ± 6.83	308.21 ± 12.1	NS	302.53 ± 8.64	315.26 ± 7.12	NS
(min)	n	32	39		38	24		37	38	
C	s_C	122.72 ± 11.07	168.74 ± 10.57	**	140.83 ± 30.09	170.31 ± 15.94	NS	169.64 ± 10.31	177.46 ± 13.36	NS
(min)	n	32	39		38	24		37	38	
F	s_F	45.31 ± 9.86	63.89 ± 11.56	NS	85.72 ± 11.51	47.87 ± 10.84	NS	58.52 ± 12.24	35.73 ± 9.05	*
(min)	n	23	30		28	17		25	22	
G	s_G	137.79 ± 15.65	69.11 ± 8.37	***	97.63 ± 12.17	84.13 ± 17.07	NS	90.31 ± 14.9	89.15 ± 12.46	NS
(min)	n	31	36		36	20		30	35	
E1	s_E1	19.36 ± 4.5	14.69 ± 2.05	NS	13.32 ± 2.61	6.84 ± 1.41	*	5.61 ± 1.36	7.33 ± 1.39	NS
(min)	n	16	26		22	17		18	24	
E2	s_E2	64.27 ± 50.43	22.70 ± 13.71	NS	57.65 ± 41.35	87.14 ± 35.25	NS	107.33 ± 46.49	88.79 ± 28.69	NS
(min)	n	4	2		3	8		6	13	

The asterisk * indicates a significant difference between the two treatments (-N and +N) (Mann–Whitney *U* tests, * $P<0.05$; ** $P<0.01$, ***: $P<0.001$). Pr: probing phase, C: stylet pathways in plant tissues except phloem and xylem, F: derailed stylet mechanics, G: active xylem sap ingestion, E1: salivation in phloem elements, E2: passive phloem sap ingestion.

Figures caption

Fig. 1 Performance parameters of *R. maidis* developing on three species of Miscanthus without (-N) or with (+N) nitrogen input. Box-plots show median (*line*), 25-75 % percentiles (*box*), 10-90 % percentiles (*whisker*) and outliers (*dots*). a) Intrinsic rate of population increase (r_m) of *R. maidis*. b) Weight of eight day-old *R. maidis* aphids. The asterisk * indicates a significant difference between the two treatments (-N and +N) for a plant host species and letters indicate significant differences between plant species associated with lsmeans (lowercase letters for non-treated plants, capital letters for treated plants) (* *P*<0.05, ** *P*<0.01, ***: *P*<0.001).





b)
M. sinensis
M. sacchariflorus
M. x giganteus
Mean weight of R. maidis (µg)
400
300
200
100
0
NS
***
**
a
C
ab
A
b
B
-N
+N
-N
+N
-N
+N

534