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Does fertilizer type drive soil and litter macroinvertebrate communities in a sugarcane agroecosystem? Evidence from a 10-year field trial

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ABSTRACT

Macroinvertebrates play a central role in processes supporting soil fertility. In the framework of the ecological intensification of agriculture, the choice of management practices should be guided by their ability to support these beneficial organisms supplying ecosystem services. This study aims at investigating the specific effect of partial substitution of synthetic fertilizers by locally produced organic fertilizers at a similar level of major nutrient inputs on macroinvertebrate abundance and diversity in sugarcane agroecosystems, on a Nitisol. Invertebrates visible to the naked eye were sampled in 2013, 2016, 2019 and 2023 using the standardized TSBF method on a long-term experimental field trial in Réunion island. The individuals were identified and soil samples were analyzed for physico-chemical properties. Despite the low response of macroinvertebrates to the fertilizer type, total macroinvertebrate abundance increased over time, especially isopods and earthworms. The input of organic carbon via the return of litter to the soil surface and the root turnover after each harvest enables soil macroinvertebrates to be more abundant even after replanting tillage. Mulching and root turnover are therefore important levers to consider for promoting macroinvertebrates in sugarcane agroecosystems.

1. Introduction

Macroinvertebrates play a central role in processes supporting soil fertility, such as decomposition of organic matter (OM) and improvement of soil structure (Lavelle et al., 2006; Rossi and Blanchart, 2005), and more generally to support the sustainability of agriculture (Lavelle et al., 2022). Some of them (ants, termites, earthworms) are often referred to as ecosystem engineers, as they modify and regulate the availability of resources for other organisms, perform a physical transformation of the environment and participate in building up soil structure (Jones et al., 1994). As such, they interact strongly with other biological communities in the soil, particularly the microbial community. Soils host a number of macroinvertebrates, with earthworms, termites and ants, the most commonly studied groups (Jiménez and Decaëns, 2006; Jouquet et al., 2006). However, saprophagous macroarthropods such as woodlice, diplopods and some beetles also directly and indirectly (through stimulation of soil micro-organisms) enhance OM decomposition (Byzov et al., 1998; Coleman et al., 2004; Lavelle and Spain, 2002; Wolters, 2000).

In order to achieve a sustainable agroecosystem aiming at

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maximizing yield while supporting biodiversity, one key aspect is the conservation of soil organic matter (SOM) (Diacono and Montemurro, 2011; Powlson et al., 2011). Indeed, SOM plays an important role in soil functioning by influencing biological, chemical and physical soil processes (Carter, 2002; Stockmann et al., 2015). The application of organic fertilizers is one of the most effective strategies to maintain and restore SOM content and soil fertility (Af, 2016; Paul et al., 1996; Rudrappa et al., 2006; Tang et al., 2022). Despite the efficiency of synthetic fertilizers to improve crop yields, their excessive use can lead to environmental damage by contributing to the release of greenhouse gases and altering soil fertility, notably by decreasing soil pH, organic matter content and beneficial organisms (Chandini et al., 2019; Pahalvi et al., 2021). In addition, synthetic fertilizers, which are usually imported and expensive, generate numerous negative impacts on the environment, including eutrophication, greenhouse gas emissions and SOM losses (Chandini et al., 2019; Pahalvi et al., 2021). The substitution of synthetic fertilizers with organic fertilizers produced locally from organic residues is being considered to increase agriculture sustainability, promote resource recycling and circular economy (Eyhorn et al., 2019; Wassenaar et al., 2014). However, agroecosystems using only organic fertilizers struggle to achieve high yield levels (Seufert et al., 2012). The joint use of synthetic and organic fertilizers offers a promising tradeoff between achieving acceptable yields and reducing environmental damages (Chen et al., 2018; Tang et al., 2022).

Achieving agricultural sustainability is a topical issue for high-potential crops, such as sugarcane. Native to Southeast Asia, sugarcane (*Saccharum officinarum*) is considered both as a food and bioenergy crop (Rott, 2018). This C4 plant is now well established as the most important sugar source worldwide, with over 90 tropical and subtropical producing countries (Moore and Botha, 2013). The optimization of its fertilization is a major challenge. Indeed, due to high yield potential, sugarcane cultivation is very demanding in terms of nutrients, and their valorization during plant development remains low (Moore and Botha, 2013). Numerous studies showed that sugarcane recovers less than half the nitrogen (N) from mineral fertilizers applied annually (Franco et al., 2011; Kingston et al., 2008). Part of this low efficiency of N valorization by the plant is linked to losses through leaching, denitrification, and volatilization (Moore and Botha, 2013).

The repetitive application of organic fertilizers, alone or in combination with synthetic fertilizers, has been shown to favor soil macroinvertebrate communities (Alves et al., 2008; Betancur-Corredor et al., 2023; Edwards and Lofty, 1982; Lavelle et al., 2006). Indeed, organic fertilizers represent an additional food source (Bertrand et al., 2015; Edwards and Lofty, 1982; Houot et al., 2014; Timmenga, 1990). They also provide a habitat for macroinvertebrates, whether beneficial or pest (Abdallah et al., 2016; Ayuke et al., 2019; Mashavakure et al., 2021; Tauro et al., 2021; Whalen et al., 1998). This is the case, for example with farmyard manure (cow and poultry), which provides habitat for foraging and oviposition by black corn beetles (Abdallah et al., 2016). Other studies on pests such as stem borers tend to show that high rates of nitrogen can favor these pests on sugarcane, thus increasing damage and yield losses (Goebel et al., 2018). Macroinvertebrate communities respond differently to organic resources, depending on intrinsic factors (diet and development cycle) and extrinsic factors (precipitation) (Tauro et al., 2021). Tessaro et al. (2013) showed that the use of organic fertilizers such as pig manure is favorable to macroinvertebrate diversity and richness, but becomes a limiting factor at high doses for ant and beetle communities. Alves et al. (2008) also suggested that the systematic use of this type of effluent can lead to the depletion of the ant community through the progressive modification of physical-chemical characteristics of the soil. According to previous authors, a combination of synthetic and organic fertilizers could offer a more balanced environment for macroinvertebrates than a unique fertilizer source (Ayuke et al., 2011). Most studies nevertheless investigated the effect of fertilization type on macroinvertebrate communities without balancing the input of each major nutrient (notably of N, P and K), thereby questioning if the observed effects were due to fertilization type or unbalance between nutrients added. In addition, numerous studies focused on comparing the effect on macroinvertebrates of a set of agricultural practices (conventional vs. organic), which typically included different fertilization types but also different tillage and pesticide application practices (Coulis, 2021). The specific effect of a partial substitution of synthetic fertilizers by different organic fertilizers within a given agroecosystem has been little studied and in particular in the context of sugarcane agroecosystems.

Consequently, this study aims at investigating the specific effect of partial substitution of synthetic fertilizers by locally produced organic fertilizers at a similar level of major nutrient inputs on the abundance and diversity of macroinvertebrate communities in sugarcane agroecosystems. We hypothesized that this fertilization strategy, compared to a single application of synthetic fertilizers, would increase (a) the total abundance of macroinvertebrates, and in particular that of the saprophagous trophic group linked to the decomposition of organic matter and (b) the diversity of taxonomic orders.

2. Materials and methods

2.1. Context of the field trial

This experiment is part of the SOERE PRO environmental research program. This Observatory for Experimentation in Environmental Research was implemented in the early 2000s to assess the agronomic effects and potential risks following repeated applications of organic fertilizers from urban and agricultural activities (sludge, compost, and manure) and subjected to various treatments (none and composting) (INRAE, 2023). It forms a network of five highly instrumented sites (three in mainland France, one in Réunion, and one in Senegal) and three other sites with little or no instrumentation worldwide (two in mainland France and one in Burkina Faso). These trials have the following main objectives:

- To study the cycles of major elements (carbon, nitrogen, phosphorus, and potassium);
- To quantify the flow of contaminants to the soil, water and plant;
- To measure gas emissions (CO_2, N_2O, NH_3) to the atmosphere;
- To monitor the evolution of microbiota, soil fauna and pathogenic organisms.

2.2. Description of the field trial

The field trial, set up in 2014, was located on the research station of La Mare, Sainte-Marie, on the northern slope of the Piton des Neiges volcano at 60 m above the sea level, on Réunion Island (20°54’12.2’’S, 55°31’46.6’’E). Mean annual precipitation and temperature at the research station are 1 650 mm and 25°C, respectively (Météo-France, 2023). The soil is a hypereutric Nitisol (FAO, 2015), exhibiting over the soil profile (ca. 1 m depth) 41 ± 6 % of clay, 15 ± 6 % of sand, an organic C content varying with depth from 21 to 4 g kg^{-1}, a pH of 6.2 ± 0.2, and a bulk density of 1.32 ± 0.07 kg dm^{-3}. Sugarcane is Réunion’s main crop as it occupies ca. 50 % of the utilized agricultural area which represents 21 000 ha for a sugarcane annual production of 1.5 million tons, with an average 13 % saccharose content. Nitisol occupies approximately 10 % of the sugarcane area (Bravin et al., 2019) and is one of the best soil types in Réunion for achieving high sugarcane yield, due to its high inherent fertility and its location on low-slope areas suitable for agricultural mechanization.

Before starting the field trial, the soil was plowed to a depth of 30–40 cm. Soil properties were homogenized by planting a cover crop of a *Brachiaria sp.* for 1 year. Following the removal of this grass cover with glyphosate and a second plowing at a depth of 30–40 cm, the field experiment began in February 2014. This involved delineating the experimental plots and planting viable bud setts of the local variety

R579 sugarcane. The field trial was a single factor randomized block design, with five fertilization treatments, replicated 5 times, i.e. 25 experimental plots of 294 m^2 each. Each plot contained six sugarcane rows of 28 m long, with 1.5 m spacing between rows. Sugarcane stalks were harvested every year in October or November, and all the litter was left on the soil surface. After the 7th harvest, in 2021, 70 % of the sugarcane litter was exported in bales and sugarcane stumps were destroyed and incorporated within the soil by a 20–30 cm depth ploughing with several deep cultivator passings over a week. New viable bud setts of the R579 variety were then planted for starting a similar, new cropping cycle.

Targeting an annual sugarcane yield between 120 and 140 t ha^{-1} (raw matter basis, RM) and based on the nutrient requirements of the sugarcane variety grown in Réunion (Fillols and Chabalier, 2007), each experimental plot received in average, and regardless of fertilizer type, 50, 150 and 215 kg (ha y)$^{-1}$ of N, P, and K, respectively. The soil was also limed and each experimental plot received an average of 215 kg Ca (ha y)$^{-1}$. The five fertilization treatments were: (i) an annual application of imported synthetic fertilizers (thereafter referred as to SF) as urea (subdivided in two applications per year), rock phosphate soluble at 75 % in 2 % formic acid, K chloride or sulfate, and Ca-Mg lime; (ii) an annual application at 2.7 ± 0.5 t RM ha^{-1} of an anaerobically-digested sewage sludge, dried, then limed, produced in Réunion by Runéo (Veolia Water) at the waste water treatment plant of Sainte Marie, and monitored for chemical (inorganic and organic) and biological contaminants (Bourdat-Deschamps et al., 2017; Laurent et al., 2023; Munoz et al., 2022) and supplemented with synthetic fertilizers as necessary and mainly driven by phosphorus from 2021 (thereafter referred as to SS1); (iii) one application every four years at 10 ± 4 t RM ha^{-1} for the first seven years and then an annual application at 6 t RM ha^{-1} of the same sewage sludge than SS1 treatment, supplemented with synthetic fertilizers as necessary and mainly driven by nitrogen from 2021 (thereafter referred as to SS2); (iv) an annual application at 73 ± 23 t RM ha^{-1} of a pig slurry produced in Réunion and supplemented with synthetic fertilizers as necessary (thereafter referred as to PS), and (v) one application every four years at 12 ± 6 t RM ha^{-1} of a poultry litter produced in Réunion and supplemented with synthetic fertilizers as necessary (thereafter referred as to PL). The physico-chemical properties of organic fertilizers and the substitution rate of N, P, K, and Ca from imported synthetic fertilizers by organic fertilizers are given in Table 1.

2.3. Soil sampling and analysis

Soil samples were collected routinely each year before fertilization. In each plot, soil cores (Ø 5 cm) were taken along a diagonal transect at 0–10, 10–20 and 20–40 cm depth, then mixed and homogenized by passing them through a 2 mm sieve to remove above-ground plant debris, roots and stones. In the study, we used the soil samples from 2013 (collected after removing *Brachiara sp.* cover and second ploughing, and before cane plantation and first fertilizer application), 2015, 2018 and 2022. Soil physico-chemical properties were determined by the routine soil-testing laboratory of Cirad in Réunion (methods described in SI 1). We calculated 0–30 cm depth values using a depth-weighted median (as bulk density did not vary significantly over soil horizons). At the start of the field trial, pH H_2O, organic C, available P (P_{avail} with the Olsen-Dabin method), cation exchange capacity (CEC), exchangeable K (K_{exch}), Na (Na_{exch}), Ca (Ca_{exch}), and Mg (Mg_{exch}), and total N (N_{tot}) respectively, were 6.1, 1.6 g 100 g^{-1}, 75.3 mg kg^{-1}, 9.6, 0.38, 0.2, 6.3, and 2.6 cmol$_+$ kg^{-1}, and 1.43 g kg^{-1}, respectively. There were no significant differences in physico-chemical properties between plots in 2013.

2.4. Macroinvertebrate sampling, identification, and community indices calculation

Litter and soil macroinvertebrates were sampled in the field

Table 1

Physico-chemical properties of the organic fertilizers used on the SOERE PRO Réunion field trial (SS1 = sewage sludge 1, SS2 = sewage sludge 2, PS = pig slurry, PL = poultry litter) and substitution rate of nitrogen (N_{SR}), phosphorus (P_{SR}), potassium (K_{SR}) and calcium (Ca_{SR}) from imported synthetic fertilizers by organic fertilizers. Data for physico-chemical properties are interannual means. Units are given in dry mass (DM) equivalent.

	DM	pH_{H2O}	C_{org}	N_{tot}	P_{tot}	K_{tot}	Ca_{tot}	Mg_{tot}	Cd_{tot}	Cr_{tot}	Cu_{tot}	Ni_{tot}	Pb_{tot}	Zn_{tot}	N_{SR}	P_{SR}	K_{SR}	Ca_{SR}
	%			g kg^{-1}					mg kg^{-1}						%			
SS1	92 ± 1	11.4 ± 0.7	330 ± 20	51 ± 4	27 ± 2	3 ± 2	89 ± 16	5.3 ± 0.8	0.62 ± 0.08	67.0 ± 10.4	202 ± 14	33 ± 4	16.0 ± 6.0	606 ± 83	36	98	3	88
SS2	87 ± 10	11.0 ± 2.0	300 ± 30	49 ± 8	25 ± 3	4 ± 3	78 ± 30	6.0 ± 2.0	0.70 ± 0.04	87.0 ± 18.2	208 ± 1	47 ± 12	22.0 ± 10.0	613 ± 100	21	82	5	61
PS	3 ± 2	7.8 ± 0.1	310 ± 60	29 ± 3	23 ± 5	116 ± 66	41 ± 9	16.0 ± 4.0	0.70 ± 0.30	9.0 ± 3.0	260 ± 126	12 ± 4	2.0 ± 1.0	1102 ± 447	54	97	70	48
PL	61 ± 3	7.9 ± 0.2	410 ± 10	37 ± 6	12 ± 3	36 ± 3	32 ± 14	7.4 ± 0.9	0.50 ± 0.20	9.0 ± 4.0	99 ± 9	7 ± 2	1.2 ± 0.9	462 ± 54	23	36	25	14

experiment according to the Tropical Soil Biology and Fertility (TSBF) methods (ISO, 2011). For each campaign, one location per experimental plot was sampled, i.e. five experimental plots/replicates per fertilization treatment and per year in total. First, beveled-edge metal frame of 25 × 25 cm was inserted in the upper 5 cm of soil to prevent any escape of epigeous macroinvertebrates during litter sampling. The litter collected manually within the metal frame was immediately packaged in a plastic bag. Then, the metal frame (30 cm deep) was inserted entirely and soil was gently excavated with a shovel and a crowbar over 25 × 25 x 30 cm and immediately packaged in a closed plastic basin. Directly on the field or in the very few following days, all invertebrates visible to the naked eye were sorted manually from litter and soil samples and preserved in 75° alcohol pillboxes for further identification and counting.

Taxonomic identification was at least carried out at class and order levels, except for Coleoptera and Oligochaeta (earthworms) that were identified at species level by taxonomists, and Hymenoptera (ants) that were identified at species level with a dichotomous key from Ramage (2010). Closer identification enabled to assign the individuals to five trophic groups in 2016, 2019, and 2023: 1) saprophagous; 2) saprophagous and predator; 3) geophagous and saprophagous; 4) predator, and 5) phytophagous (SI 2). Abundance (total number of individuals) was calculated per m^2, summing soil and litter organisms. Alpha diversity indexes (richness, Simpson and Shannon index) were calculated at the order level using the vegan package in R (Oksanen et al., 2007).

Macroinvertebrates were preliminary sampled in November 2013 under *Brachiaria* cover. This sampling was used to characterize the initial state of soil macroinvertebrates before first fertilizer application and sugarcane planting. In total, 1 712 specimens (including 32 specimens not assigned) were collected at the start of the field trial, with 15 % annelids and 85 % arthropods, including 47 % Hymenoptera, 16 % Arachnida, and 10 % Coleoptera. No Isopoda was sampled in this first sampling campaign. There was no significant difference in abundance (for total macroinvertebrates and for each taxa) and diversity (richness, Shannon index at order level) between the plots just before the beginning of the field trial in 2013. In 2016, 2019, and 2023, macroinvertebrates were sampled in April (at the end of the humid austral summer) in the middle of the annual cropping cycle, which favors the presence of macroinvertebrates under a well-developed sugarcane cover and with a favourable soil moisture content. During the first sugarcane planting cycle, macroinvertebrates were sampled in 2016 and 2019 on the five replicates of the five fertilizer types (1 sample per plot, 25 samples in total per year). After replanting sugarcane, all plots were sampled in 2023 except for 3 replicates of the treatments PS and PL.

2.5. Statistical analyses

The effects of time (year of cultivation) and fertilization treatment (from 2016 to 2023) were tested on soil and macroinvertebrates variables (total abundance, abundance of individual orders, trophic groups, and alpha diversity indexes) using a non-parametric (rank) test for longitudinal data, using the function f1.ld.f1() from the package nparLD (Noguchi et al., 2012). When a main effect was significant, a non-parametric multiple comparisons test was performed using pairwise.wilcox.test() function (for paired data), for the year variable, or nparcomp() function from the package nparcomp (Konietschke et al., 2015) for the fertilization treatment variable (per year). All macroinvertebrates were considered in the statistical processing, with the exception of orders (i.e. Diptera and Gastropoda) that were under-represented in the dataset (less than 1 % of the individuals). The total abundance of ants was analyzed separately, in view of their numbers and their high degree of aggregation (social behavior). Correlations between soil variables and abundance of individual taxa and functional groups were calculated using Spearman correlation tests using the corr.test() function of the psych package (Revelle, 2024). Statistical analyses were carried out with R version 4.1.2 (R Core Team, 2021). Soil and macroinvertebrates datasets used for statistical analyses are accessible via Dataverse (Jacquin et al., 2023), and the R code via Gitlab and Software Heritage (please refer to the “code linking” section). Statistical tests were considered as significant for $p < .05$.

3. Results

3.1. Effect of year of cultivation and fertilizer type on soil physico-chemical properties

While soil physico-chemical properties were homogeneous over the field trial in 2013, they evolved significantly between 2015 and 2022 due to sugarcane cropping. The year of cultivation was the main driver of soil properties, and to a lesser extent the fertilizer type. The year of cultivation had a significant effect on all soil properties (Table 2, all $p < .001$). The pH, organic C, CEC, exchangeable Ca, and exchangeable Mg increased significantly from 2015 to 2022, while reserve acidity (ΔpH) decreased. For total N, available P, and exchangeable K, a decrease was observed in 2018 compared to 2015, followed by an increase in 2022, and the opposite trend for exchangeable Na. The fertilizer type had a more limited impact, only significant for pH, exchangeable Ca and Mg ($p = .017$,.025 and <.001, respectively), and no specific trend resulting from the partial substitution of synthetic fertilizers by organic fertilizers could be identified (Table 2).

3.2. Effect of year of cultivation and fertilizer type on the abundance and diversity of macroinvertebrates

The total abundance of soil macroinvertebrates (excluding ants), and the total abundance of ants were impacted significantly by the year of sugarcane cultivation ($p < .003$ and.001, respectively) but not by the fertilizer treatment ($p = .48$ and.41, respectively) (Fig. 1). Total abundance of macroinvertebrates (excluding ants) increased over time, starting from 69 individuals m^{-2} on average in 2013, then 171, 303, and 454 individuals m^{-2} in 2016, 2019, and 2023, respectively. The same trend was observed for ants, with only 57 ants present on average in 2013, then 560, 857 and 1985 individuals m^{-2} in 2016, 2019, and 2023, respectively. Alpha diversity indexes (richness, Pearson and Shannon at order level) did not change significantly with time and fertilizer treatment (SI 3).

No significant effect of fertilization treatment was observed either at the individual order level nor at the trophic group level. On the contrary, a significant increase of abundance with year of cultivation was observed for saprophagous animals ($p < .001$), and, within this trophic group, for Haplotaxida and Isopoda ($p = .011$ and <.001, respectively) (Fig. 2). The opposite trend was observed for the effect of year of cultivation on the abundance of Coleoptera ($p = .02$).

Overall, 9 earthworm species were identified in 2016, 2019 and 2023. *Pontoscolex corethrurus* (Rhinodrilidae) and *Dichogaster bolaui* (Acanthodrilidae) were the most abundant species (SI 2). In 2023, the only year when ants were identified at the species level, the vast majority of them were represented by two species well established in Réunion: *Solenopsis geminata* and *Brachymyrmex cordemoyi* (California Academy of Sciences, 2023) (SI 4).

3.3. Relationship between soil properties and the abundance of macroinvertebrates

Most macroinvertebrate orders and trophic groups were correlated to at least one soil physico-chemical property (Fig. 3), except for the Blattodea and Coleoptera orders, and the “geophagous;saprophagous”, “phytophagous” and “saprophagous;predator” trophic groups. The reserve acidity (ΔpH) and CEC were the most significantly negatively and positively correlated properties, respectively, especially for Isopoda and saprophagous animals.

Table 2
Soil physico-chemical properties (pH H_2O, ΔpH (the difference between pH_{H2O} and pH_{KCl}), organic C (C_{org}), total N (N_{tot}), cation exchange capacity (CEC), exchangeable Ca (Ca_{exch}), Mg (Mg_{exch}), K (K_{exch}), and Na (Na_{exch}), available P (P_{avail}); mean±SD) per year of cultivation and fertilizer type (SF = synthetic fertilizers, PL = poultry litter, PS = pig slurry, SS1 = sewage sludge 1, SS2 = sewage sludge 2). Units are given in dry mass equivalent. The effect of the year of cultivation and fertilization treatment are indicated with upper- and lower-case letters, respectively.

Modality	pH_{H2O}	ΔpH	C_{org}	N_{tot}	CEC	Ca_{exch}	Mg_{exch}	K_{exch}	Na_{exch}	P_{avail}
Unit			g kg^{-1}		———cmol$_+$ kg^{-1}———					mg kg^{-1}
2015										
	A	C	A	B	A	A	A	B	A	B
SF	6.2 ± 0.2	1.3 ± 0.0	16 ± 1	1.5 ± 0.1	10.2 ± 0.7	6.2 ± 0.7	2.9 ± 0.3	0.5 ± 0.1	0.2 ± 0.1	92 ± 30
PL	6.4 ± 0.2	1.3 ± 0.0	17 ± 1	1.6 ± 0.1	11.1 ± 0.6	6.7 ± 0.3	3.4 ± 0.3	0.4 ± 0.2	0.2 ± 0.0	78 ± 24
PS	6.2 ± 0.1	1.3 ± 0.1	16 ± 1	1.5 ± 0.1	10.0 ± 0.6	5.9 ± 0.2	2.9 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	66 ± 21
SS1	6.1 ± 0.1	1.3 ± 0.0	17 ± 1	1.5 ± 0.1	10.1 ± 0.4	6.4 ± 0.6	2.8 ± 0.2	0.4 ± 0.1	0.2 ± 0.0	84 ± 18
SS2	6.2 ± 0.2	1.2 ± 0.1	17 ± 1	1.6 ± 0.1	10.9 ± 0.8	6.7 ± 0.5	3.0 ± 0.2	0.4 ± 0.0	0.2 ± 0.1	104 ± 30
2018										
	B	B	A	A	B	B	B	A	C	A
SF	6.3 ± 0.2	1.2 ± 0.1	16 ± 2	1.4 ± 0.2	11.6 ± 1.0	6.5 ± 0.8^{ab}	3.1 ± 0.2^{ab}	0.3 ± 0.1	0.3 ± 0.1	60 ± 20
PL	6.5 ± 0.2	1.3 ± 0.1	17 ± 2	1.5 ± 0.1	12.7 ± 0.8	6.9 ± 0.3^{ab}	3.6 ± 0.3^{c}	0.3 ± 0.1	0.3 ± 0.1	56 ± 20
PS	6.3 ± 0.1	1.3 ± 0.1	17 ± 2	1.5 ± 0.2	12.0 ± 0.7	6.4 ± 0.5^{a}	3.3 ± 0.1^{bc}	0.3 ± 0.1	0.4 ± 0.1	49 ± 20
SS1	6.2 ± 0.1	1.2 ± 0.1	17 ± 1	1.5 ± 0.1	11.6 ± 0.6	7.0 ± 0.3^{ab}	2.9 ± 0.2^{a}	0.3 ± 0.1	0.3 ± 0.0	65 ± 17
SS2	6.3 ± 0.2	1.2 ± 0.0	18 ± 1	1.6 ± 0.1	12.6 ± 0.5	7.4 ± 0.5^{b}	3.2 ± 0.2^{abc}	0.3 ± 0.1	0.3 ± 0.1	90 ± 26
2022										
	B	A	B	C	C	C	C	C	B	B
SF	6.3 ± 0.2^{ab}	1.0 ± 0.1	19 ± 2	1.5 ± 0.2	12.3 ± 1.3	7.0 ± 0.9^{ab}	3.3 ± 0.3^{a}	0.6 ± 0.1	0.3 ± 0.1	77 ± 21
PL	6.6 ± 0.1^{b}	1.1 ± 0.1	20 ± 3	1.7 ± 0.2	13.5 ± 1.1	7.9 ± 1.0^{ab}	3.8 ± 0.3^{ab}	0.5 ± 0.0	0.3 ± 0.1	81 ± 18
PS	6.4 ± 0.1^{b}	1.1 ± 0.1	20 ± 2	1.6 ± 0.2	12.4 ± 0.4	7.0 ± 0.3^{a}	3.7 ± 0.1^{b}	0.5 ± 0.1	0.4 ± 0.0	72 ± 11
SS1	6.2 ± 0.1^{a}	1.0 ± 0.1	21 ± 2	1.7 ± 0.2	12.0 ± 2.9	7.4 ± 1.9^{ab}	3.0 ± 0.7^{a}	0.6 ± 0.1	0.2 ± 0.1	97 ± 30
SS2	6.3 ± 0.2^{ab}	1.0 ± 0.1	20 ± 2	1.7 ± 0.2	13.2 ± 0.3	8.0 ± 0.2^{b}	3.4 ± 0.3^{ab}	0.5 ± 0.0	0.3 ± 0.0	123 ± 35

4. Discussion

4.1. Duration of sugarcane cropping rather than fertilizer type drives the evolution of soil macroinvertebrate communities

The establishment of sugarcane has modified the biological and physico-chemical properties of the soil over time. The strongest effect observed on macroinvertebrate communities was the time since the start of sugarcane cultivation. The total abundance of macroinvertebrates, ants, Haplotaxida and Isopoda, as well as the abundance of the saprophagous trophic group increased over time, while Coleoptera abundance decreased (Figs. 1 and 2). This community development can probably be explained by modifications over time in the physico-chemical properties of the soil due to the cropping system, in particular pH, ΔpH, and Corg (Fig. 3).

The organic carbon content increased progressively over time, reaching a 25 % increase in 2022 compared to 2013. This increase is not due to the OM from organic fertilizers, since there was no significant differences in the soil Corg between fertilization treatments in 2022 (Table 2). We can hypothesize that other sources of OM are involved: either dead root biomass, or sugarcane litter return to the soil surface after each harvest, and in particular the residues incorporated into the soil when a new sugarcane cycle was replanted (in 2021). The fertilization treatments were designed to produce an equivalent sugarcane biomass in all treatments. As a consequence, the OM produced by the plant and restituted to the soil, ca. 5 and 2 t C_{org} (ha y)$^{-1}$ of roots and litter, respectively, was approximately the same in all treatments as well. By comparison, the amount of imported OM by fertilization was lower than 1 t Corg (ha y)$^{-1}$ on average, whatever the fertilizer type. This input was small (i.e. less than 15 %) compared to the biomass produced and restituted to the soil. We can thus consider that C input and consequently its effect on macroinvertebrate communities was more or less the same in all fertilization treatments.

Mulching the soil surface most likely favored the development of the macroinvertebrates in the study, providing them with both a habitat and a food resource (Abreu et al., 2014; Coulis, 2021; Pasqualin et al., 2012; Portilho et al., 2011). Isopoda, the main saprophagous organisms in the trial, are the animals that benefited most from this practice, and in particular from the incorporation of litter into the soil in 2021. During straw decomposition, nutrients are released into the soil-plant system. The rapid release of K following litter incorporation may justify the significantly higher Kexch concentrations in 2022. In addition, soil organisms are known to be sensitive to disturbances associated with agricultural practices, such as frequent and deep tillage (Brennan et al., 2006; Cortet et al., 2002; Kuntz et al., 2013). So, the positive effect of litter apparently counterbalanced the presumed negative effect of replanting tillage on macroinvertebrates.

Between 2013 and 2022, we observed a 3.8 % increase of the pH, and a 23.1 % decrease of reserve acidity (delta pH), regardless of fertilizer types. These results are probably due to the maintenance liming applied every year, with Ca and Mg incorporated within sewage sludge during its processing, or complementary added along with mineral and organic fertilizers. Organic fertilizers are also known to alkalize acidic soils (Haynes and Mokolobate, 2001), thus maybe explaining why soil pH tended to be slightly higher with the application of pig slurry and poultry litter (i.e. PS and PL fertilizer type). The decrease in soil acidity reserve, and, to a smaller extent, the increase in soil pH, impacted the abundance of macroinvertebrates, especially Hemiptera, and saprophagous organisms, such as Haplotaxida and Isopoda. The observed correlation between soil acidity and macroinvertebrates is consistent with the literature (Kuperman, 1996). Springett and Syers (1984) showed under controlled conditions that both pH increase and soil liming (Ca input) increased earthworm cast production. Auclerc et al. (2012) showed at a catchment scale that liming could impact (by both pH increase and Ca input) the abundance of macroinvertebrate taxa either positively or negatively. These results suggest the need to deepen our understanding of the mechanisms behind the effect of soil acidity on macroinvertebrate communities.

As for the observed decrease in Coleoptera abundance, we hypothesize that as the ant population increased, there was more predation placed on the beetle population. This can argue in favor of stronger pest regulation thanks to the increase in soil predators such as ants.

Contrary to initial expectations, the fertilizer type had little effect on macroinvertebrate communities. In addition, sewage sludge, often considered to cause negative effects on soil biota (Cesar et al., 2008), had no detrimental effect on macroinvertebrates or even tended to have a little beneficial effect (although not significant). In 2023, Haplotaxida order and saprophagous trophic group were more abundant under the

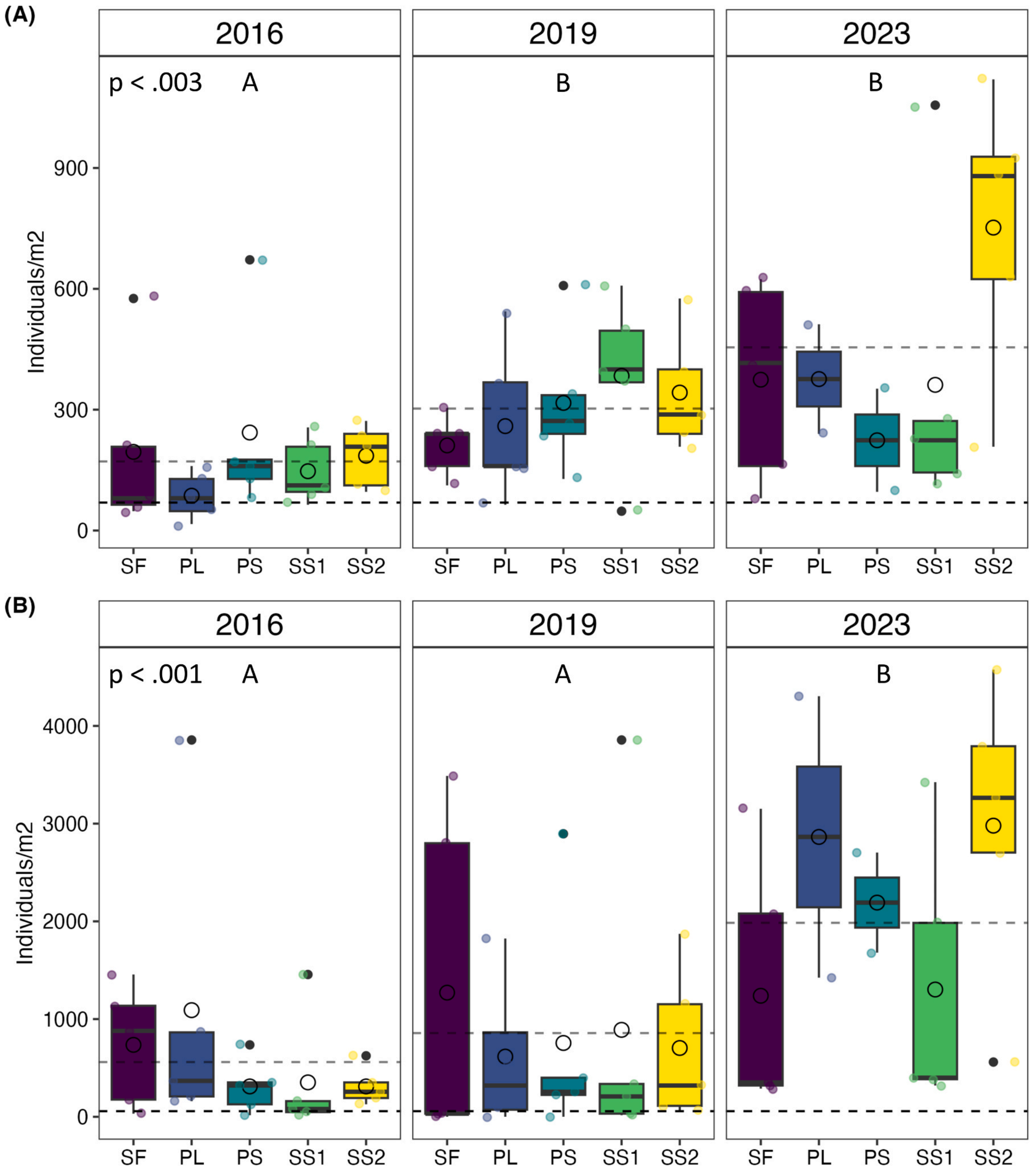

Fig. 1. Boxplots of the total abundance (individuals/m^2) of macroinvertebrates excluding ants (A) and of ants (B) per year of cultivation and fertilizer type (SF = synthetic fertilizers, PL = poultry litter, PS = pig slurry, SS1 = sewage sludge 1, SS2 = sewage sludge 2). The p-values of the year of cultivation effect, as well as the results of the post hoc tests, are indicated. The fertilization treatment effect was not significant. The black dotted lines represent the average at the beginning of the trial (in 2013) and the grey dotted lines correspond to the average per year of cultivation. Black points and empty circles correspond to outliers and average for each fertilizer type, respectively. Colored points correspond to the value of each replicate, i.e. individual plot.

higher dose of sewage sludge (SS2 treatment, approximately 3.8 t RM (ha y)$^{-1}$ in average) than under the lower dose of sewage sludge (SS1 treatment, approximately 2.7 t RM (ha y)$^{-1}$) and the synthetic fertilizer treatment (SF). These results suggest that the positive impact on macroinvertebrates of a decadal use of sewage sludge outweighs the potential ecotoxicological effects due to its content in chemical contaminants such as trace elements and organic contaminants (Huguier et al., 2015). This hypothesis is consistent with the very low concentrations of some pharmaceuticals and personal care products (PPCPs, such as fluoroquinolone and tetracycline antibiotics, carbamazepine, and diclofenac) added by the repeated applications of organic fertilizers and recovered in the soil of the Réunion field trial and two older field trials from the same observatory located in mainland France (Bourdat-Deschamps et al., 2017). The risk assessment of PPCPs ecotoxicity to soil organisms including macroinvertebrates was estimated to be low at such agronomically-relevant fertilizer application rates. Regarding trace elements, and more particularly copper (Cu) and zinc (Zn), which are the most worrying trace elements from an ecotoxicological perspective, the bioavailability of Cu and Zn added by a decade of repeated applications of organic fertilizers (including sewage sludge) was shown to be similar to that of the synthetic fertilizer treatment (SF). This result was driven by the naturally high Cu and Zn pedogeochemical background in Réunion and the mitigation effect of organic fertilizers (through increasing soil pH and organic matter) on Cu and Zn

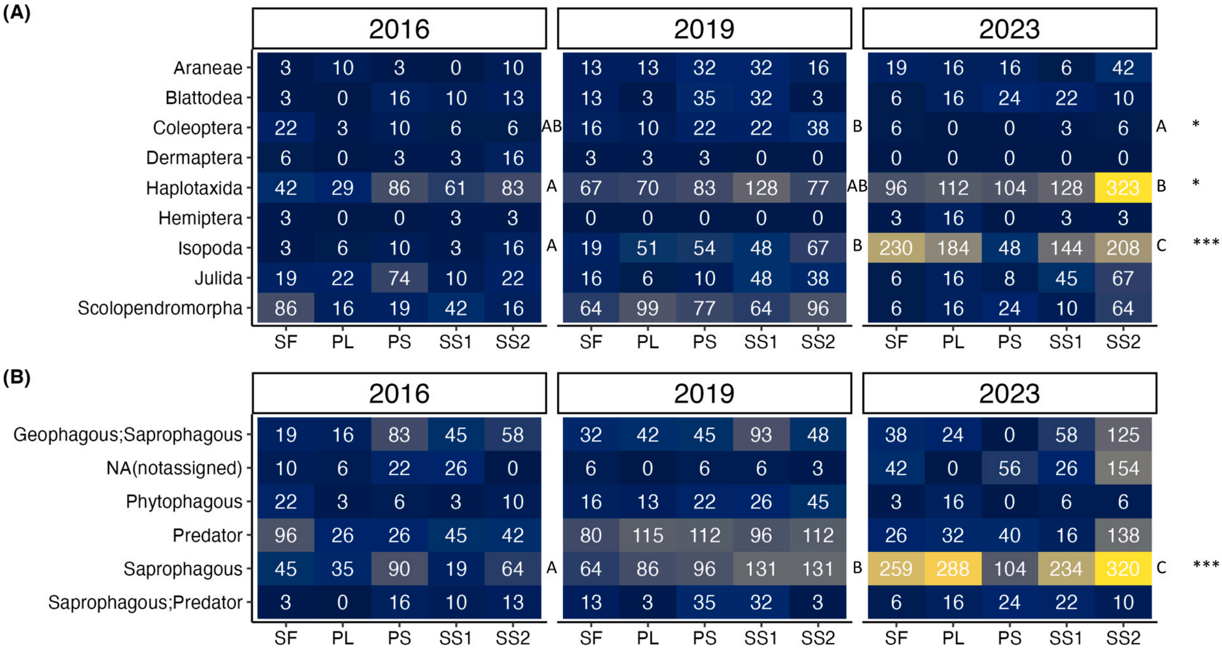

Fig. 2. Heatmap of the average total abundance (individuals/m^2) of macroinvertebrates per orders (A) and trophic groups (B) per year of cultivation and fertilizer types (SF = synthetic fertilizers, PL = poultry litter, PS = pig slurry, SS1 = sewage sludge 1, SS2 = sewage sludge 2). The effect of the year of cultivation is indicated by upper-case letters (* p < .05, ** p < .01, and *** p < .001). The effect of fertilization type was not significant.

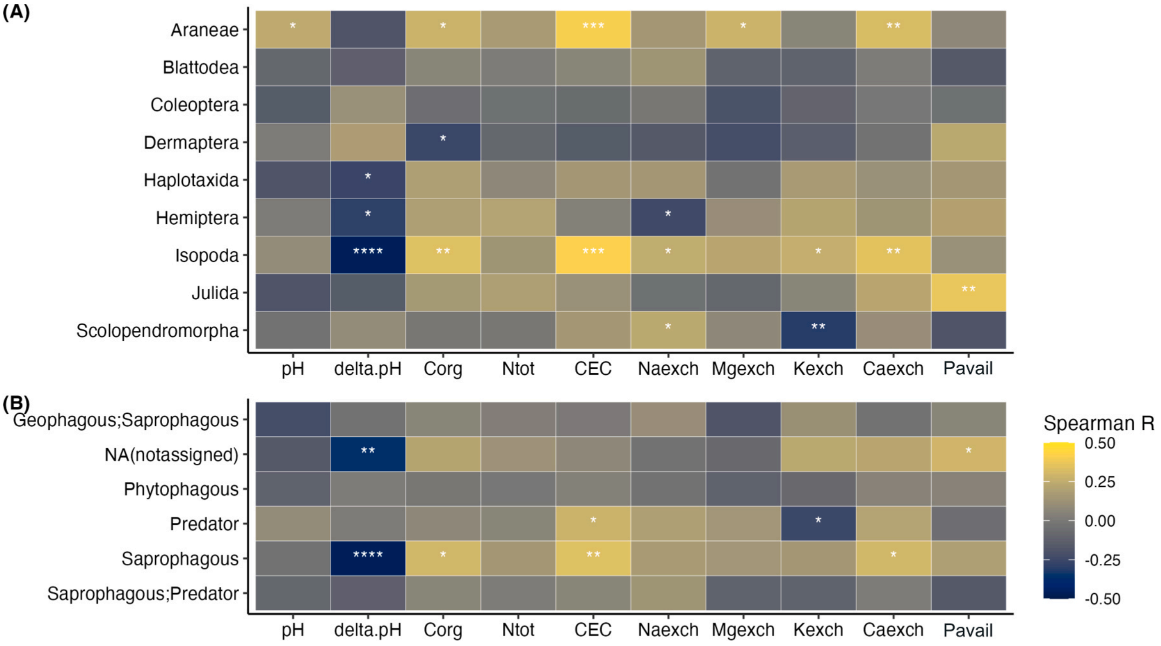

Fig. 3. Correlation matrix between soil physico-chemical properties (pH H_2O, delta pH (the difference between pH_{H2O} and pH_{KCl}), organic C (C_{org}), total N (N_{tot}), cation exchange capacity (CEC), exchangeable Ca (Ca_{exch}), Mg (Mg_{exch}), K (K_{exch}), and Na (Na_{exch}), available P (P_{avail})) and abundance of individual taxa (A) and trophic groups (B). * ** * p < .0001, * ** p < .001, * * p < .01, and * p < .05.

availability in soil (Laurent et al., 2023).

4.2. Earthworm and ant communities dominated by introduced species

In our study, the majority of earthworm and ant species observed are introduced ones. *S. geminata* is the most abundant ant species (SI 4), confirming the high density of this species in the anthropogenic environments of eastern Réunion (Blard, 2006). *S. geminata* is one of the most invasive and destructive ant species at global level (Ward and Harris, 2005). *P. corethrurus*, one of the two most abundant earthworm species in the study (SI 4), is also the most widespread earthworm species in tropical zones and in a wide range of habitats (Taheri et al., 2018), due to its great ecological plasticity (Sakai et al., 2001). This cosmopolitan species seems to proliferate in disturbed habitats such as cropping and market gardening systems (Marichal et al., 2010). The presence of this species in the sugarcane agroecosystem is rather welcomed since its feeding activity contributes to increasing the biomass and activity of microorganisms (Bernard et al., 2012) and to increasing nutrient availability to plants (van Groenigen et al., 2014).

Our results are in line with previous studies showing that Réunion's fauna is a mix of introduced and native species (Legros et al., 2020). The ants described in Réunion are mainly composed of introduced species (California Academy of Sciences, 2023). Introduced species come mainly from human activities such as intense international trade and housing transformations (Blackburn et al., 2016), and are favored by particular conditions, including high input agroecosystems (Fragoso et al., 1997). According to the study by Fragoso et al. (1997) on tropical earthworms, native species are frequently found in agroecosystems of tropical countries where inputs are low. These conditions would limit the expansion of introduced species. On the contrary, sugarcane agroecosystems that are heavily dependent on fertilizer inputs could encourage the development of introduced species. Biodiversity loss in islands is mainly linked to biological invasion (Myers et al., 2000). Introduced species can easily compete for resources and lead to a loss of genetic diversity by hybridizing with native species (Legros et al., 2020).

5. Conclusion

This study investigated the abundance of macroinvertebrate communities in litter and soil of a sugarcane agroecosystem as a function of fertilizer type, i.e. partial substitution of imported synthetic fertilizers by locally produced organic fertilizers, applied at a fixed level to fuel a high yield target. The initial hypothesis was that the substitution by organic fertilizers would increase (a) the total abundance of macroinvertebrates and in particular saprophagous trophic group, and (b) the diversity of taxonomic orders. Contrary to this initial hypothesis, the fertilizer type had little effect on macroinvertebrate communities. The main effect was due to the duration of sugarcane cropping, increasing the abundance of saprophagous animals and, within this trophic group, Haplotaxida and Isopoda. These changes in macroinvertebrate communities were probably induced by evolutions in soil physico-chemical properties, mainly soil acidity decrease, and sugarcane litter and root OM inputs to the soil rather than from organic fertilizers. If soil acidity improvement is the focus of many studies, litter contribution and root turnover on invertebrate communities, especially in the context of monocropping, is much less investigated and should be the subject of dedicated studies.

It is also important to note that the ant and earthworm communities found in the trial are composed of very few species native to Réunion. Although the behavior of some invasive species is documented, the lack of literature for most of the species found is a limitation to understanding their impact within the sugarcane agroecosystem.

CRediT authorship contribution statement

François-Régis Goebel: Writing – review & editing, Validation, Resources, Methodology. **Charles Detaille:** Writing – review & editing, Validation, Resources, Investigation. **Malalatiana Razafindrakoto:** Writing – review & editing, Validation, Methodology, Investigation. **Janine Jean:** Writing – review & editing, Validation, Resources, Methodology, Investigation. **Estelle Jacquin:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Investigation, Formal analysis, Data curation. **Eric Blanchart:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Marie-Liesse Vermeire:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Formal analysis, Data curation, Conceptualization. **Matthieu N. Bravin:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: E. Jacquin, M. L. Vermeire, and M. N. Bravin reports financial support was provided by Conseil Régional de La Réunion. E. Jacquin, M. L. Vermeire, and M. N. Bravin reports financial support was provided by French Government Ministry of Agriculture and Food Department of Forest Health. E. Jacquin, M. L. Vermeire, and M. N. Bravin reports financial support was provided by European Regional Development Fund. E. Jacquin, M. L. Vermeire, and M. N. Bravin reports financial support was provided by Veolia Water. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data linking

The dataset supporting this analysis is freely available on Dataverse: Jacquin, E., Vermeire, M.-L., Detaille, C., Bravin, M., 2024. Replication Data for: Does fertilization type drive soil and litter macroinvertebrate communities in a sugarcane agroecosystem? Evidence from a 10-year field trial. CIRAD dataverse, v1 DOI : https://doi.org/10.18167/DVN1/VDUXQP.

Private URL: https://dataverse.cirad.fr/privateurl.xhtml?token=e135e2b4-53ba-4ad3-974c-96fd81af2941

Code linking

The code used for data analysis is freely available

on GitLab:
https://gitlab.cirad.fr/recyclageetrisque/macrofaune_soere_pro_reunion
on SoftwareHeritage:

SWHID: swh:1:dir:a408f931a8ebb506a7caf815560a1aa04df2a777;
origin=https://gitlab.cirad.fr/recyclageetrisque/macrofaune_soere_pro_reunion;visit=swh:1:snp:3c2b44cf1e6beb3cbd57d63323b8e6957672eba5;anchor=swh:1:rev:e4228e1c18e521f933dd49b42750296d76857d06;
visit=swh:1:snp:01c287d9d50b05491d3b973cbff963fb90afbf75

To cite the code

Vermeire, M.-L. (2024). Macroinvertebrates Diversity Analysis - SOERE PRO REUNION.

Archived in software heritage

[https://archive.softwareheritage.org/swh:1:dir:a408f931a8ebb506a7caf815560a1aa04df2a777;origin=https://gitlab.cirad.fr/recyclageetrisque/macrofaune_soere_pro_reunion;visit=swh:1:snp:3c2b44cf1e6beb3cbd57d63323b8e6957672eba5;anchor=swh:1:rev:e4228e1c18e521f933dd49b42750296d76857d06]

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.109431.

Data availability

The data and code will be shared in open access on dataverse and GitLab

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