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RESEARCH

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Challenge of transition: the history of a case study involving tropical fruits polyculture stimulated by humic acids and plant-growth promoting bacteria

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Abstract

Background: Innovative technologies are required in agricultural production to eliminate the environmental risk generated by the intensive use of fertilizers and pesticides. Soil organic matter is a keystone for the transition towards sustainable production. However, it is not an easy task to increase soil organic matter in highly weathered soils without considerable resources and energy. Here, we highlighted the role of biological inputs in plant adaptation to low fertility and water scarcity. The direct use of humic acids and plant growth-promoting bacteria on plants can modify the root architecture systems, including surface area and roots length, thus allowing greater soil exploration.

Material and methods: Within a socio-historical perspective of concepts and research methods, a case study is presented on the effects of humic acids applied together with plant-growth promoting bacteria, as an efficient tool for supporting the transition to more suitable production system. We implemented this natural ecological approach onto a polyculture system with different tropical fruits (banana, passion fruit, papaya and pineapple) and evaluated crop yields.

Results: We observed increases of around 50 and 90% in banana and papaya yield, respectively, and 25% in passion fruit productions, with significantly greater yields maintained over four production cycles. No effect was observed in ananas production probably due to the large shading level in the area.

Conclusion: The biostimulant formulated with endophytic diazotrophic bacteria and humic acids represents a low-cost technology that enhances crop yields and can play an important role in promoting a transition process towards sustainable agriculture.

Keywords: Bioinputs, Humic substances, Microbial technologies, Bioeconomy, Eco-friendly practices

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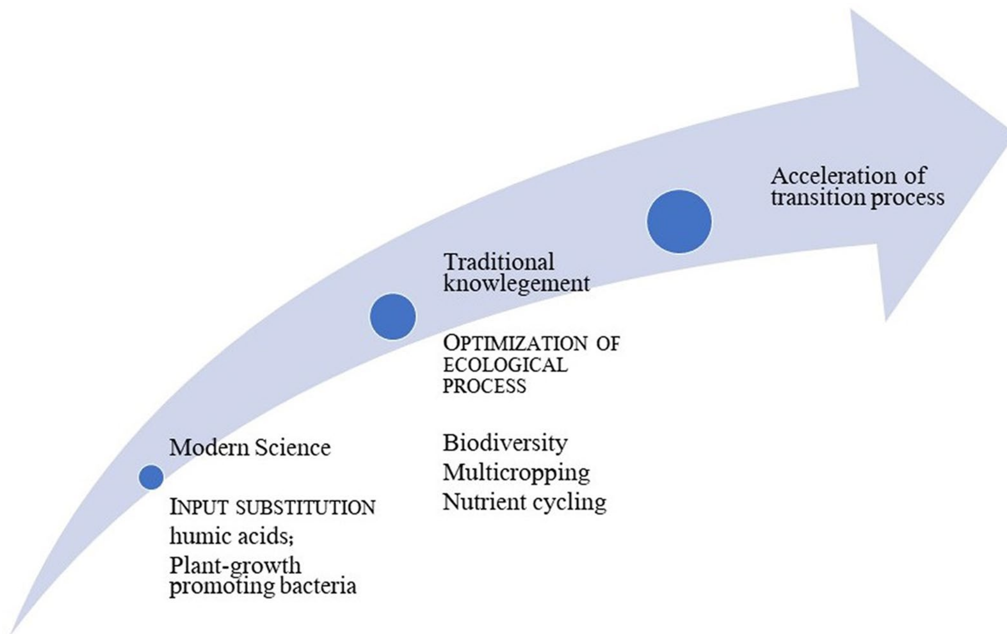
Graphical Abstract



Introduction

Conventional agriculture maintains high crop yields based on chemical inputs, including fertilizers and pesticides. It is urgent to find transitional means to sustain productivity by implementing a more sustainable agriculture and reducing industrial agrochemical products. One strategy resides in exploiting the plant growth promoting potential [1] of humic substances (HS) which can be obtained from local resources. In recent years, the interest in applying soluble HS at low concentration to stimulate plant growth has progressively grown. This trend reflects the increasing consensus among different stakeholders, such as distributors, researchers, extension officers and farmers, on the impact of humic matter as a useful product based on well-established evidence of field trials [2]. Soil organic matter (SOM) is responsible for preserving soil functions, improving plant nutrients status, regulating biogeochemical cycles, and mitigating greenhouse gases emission [3]. Due to its crucial role in sustaining life, no other issue related to soil chemistry changed its fundamental concepts in such a short time as humus chemistry [4, 5]. The understanding of the supramolecular nature of soil humus [6] has paved the way to the unravelling of its molecular composition [7, 8] and, consequently, to the intensifications of investigations on the effects of HS as a plant growth promoter and on the structure–bioactivity relationships [9, 10].

A number of the scientific reports on the application of HS as plant growth promoters were recently published with different approaches [11–21]. The present work offers a case study of the chronological perspective of concepts and research methods related to the physiological effects of HS conducted in the laboratory of *Núcleo de Desenvolvimento de Insumos Biológicos para Agricultura* (Nudiba). As a self-assessment, the authors are anyhow aware that inputs substitution does not always guarantee improved agricultural outcomes. In fact, nowadays the conventional industry already provides biological inputs [22]. However, the central debate in this context should focus on the technological dependence of farmers and how to overcome the ecological limitations encountered at the first step of a transition towards more sustainable agriculture. In fact, humic products can be easily handled by farmers at field sites and contribute to overcome the typical complications, such as changes in plant nutrition, rise in pests and diseases, limitation of water resources, that may accompany the onset of a transition process when associated to appropriate and selected strains of beneficial microorganisms.

Historical perspective

The aim of research at Nudiba has been the better understanding of the effects of soil management on humus content and quality [23–26]. The turning point spurred after

Table 1 Typical chemical properties and texture of surface layer (0–0.2 m) of *Argissolo Amarelo* soil [Ultisols] in an agrarian settlement in the north of Rio de Janeiro State

Site	pH $_{H_2O}$	H + Al	Al^{3+}	P	K^+	Ca^{2+}	Mg^{2+}	Na^+	C	N	CEC	Sand	Silt	Clay
		$cmol_c$ kg^{-1}		mg kg^{-1}	$cmol_c$ kg^{-1}				(%)	(%)	$cmol_c$ kg^{-1}	%		
#1	5.4	7.7	0.3	8	0.10	2.4	1.9	0.10	2.4	0.2	12.2	54.0	10.0	36.0
#2	4.5	12.1	2.5	3	0.12	0.5	0.5	0.09	2.6	0.2	13.3	50.0	11.0	39.0
#3	5.0	9.7	0.9	2	0.15	2.3	0.8	0.04	2.2	0.2	13.0	56.0	7.0	37.0
#4	5.1	8.3	1.8	2	0.15	1.0	0.5	0.02	1.8	0.2	11.5	49.0	8.0	38.0

many low-endowed farmers began to refer to Nudiba's lab and asked to enhance crop yields in their degraded fields, where sugarcane was historically produced for over 300 years. These farmers occupied marginal and degraded lands of abandoned sugarcane plantations after the bankruptcy of sugarcane mills in the North of Rio de Janeiro state, Brazil, at the end of 1990s.

During the colonial period, more than 400 old sugarcane mills were established in this region. The current activity reached its climax in the early 1970s during the first global oil crisis when 27 operating mills had to face a progressive closure. In the years 2000, ten sugar and alcohol mills were still in operation using more than 250,000 ha and milling above 10 million tons of sugarcane per year [27]. Today only two mills are left in activity.

The reasons for this collapse are diverse and complex and were the subject of many academic studies. Although it is not our intention to enter this issue, we remark the inherent social and economic damages, such as the final lay-off of field and factory workers with very low or absent education. Contrary to other sugarcane regions in Brazil where the process of the conservative technological modernization had taken place, the North of Rio de Janeiro persisted in an obsolete manual harvest after the burning of sugarcane fields. The evident negative effects of this management practice on soil chemical properties, including SOM quality and content, were thoroughly studied [28–30].

Lands with low natural fertility of Oxisols, Ultisols and Alfisols soil types were abandoned, and some areas were occupied by former workers of the sugarcane mills and by lumpen from urban fringe organized by a social movement of landless workers (MST). They initiated crop production quickly after sugarcane withdrawal without any soil rest by using traditional subsistence techniques without chemical inputs. After different periods (three, four or ten years) of waiting for the final judicial decision, an agrarian reform settlement was acknowledged, and new farmer families became landowners and began to benefit from official programmes of technical and financial assistance. Some peasants grew eager to implement agroecological concepts and orient their practice to enhance productivity. In particular, Nudiba was specifically asked to design technologies to increase plant root systems devoted to enhance soil volume exploitation and optimize the exploitation of the scarce resources, i.e., nutrients and water.

The main edaphic constraints, according to the Fertility Capability soil Classification system [31], that were observed in soils developed on the tertiary sediment named "Barreiras" in the North of the Rio de Janeiro State can be summarized as: (i) the presence of dry seasons longer than three months—April to August; (ii) low nutrient reserves within soil minerals; (iii) high risk of soil erosion by run-off water due to abrupt textural gradients and/or steep slopes in a typical half-orange shape; (iv) low pH and aluminum (Al^{3+}) toxicity. Table 1 shows some typical results of soil chemical analyses. It is also noticeable the low cation exchange capacity (CEC) and low available phosphorus content. Nitrogen is a serious limiting factor.

The most appropriate measure to overcome these constrains is to enhance SOM content. Plants grown on soils enriched with organic matter are less subject to stress, healthier and more productive. Soil Humus improves water-holding capacity due to its oxygen-containing functional groups that enhance bondings with water molecules [32], and stabilizes soils, thereby facilitating water infiltration and reducing run-off erosion [33, 34]. Moreover, soil organic matter is the source of nutrients like nitrogen, phosphorus and sulfur, and can reduce Al^{3+} toxicity by complexation [35].

However, it is not easy to enhance SOM content in highly weathered soils since high biological activity during all year round induces a fast organic residues mineralization. The warm but dry winter in the tropics limits nitrogen mineralization and leaching, thus causing a reduced microbial activity [36]. At the first rains, there is the "Birch effect" consisting in a flush of nitrogen mineralization, as the survived bacteria determine an immediate mineralization of organic nitrogen. When the rainy season starts, the mineralization rate increases even more. Moreover, decomposition of native organic matter is advanced further from a cropped low-fertile soil since the nutrient-limited microbial biomass requires the carbon of native organic matter to support its respiration [31].

We produced some experimental models to monitor the dynamic of available organic residues in a controlled situation and we observed that priming effect reduced organic matter content below an initial status. Only concentrations equivalent to or above 240 Mg ha^{-1} resulted in an organic carbon increase after two years in an Oxisol [37]. In fact, the fast turnover of organic matter is typical of tropical climate. Despite the poor conditions of highly weathered soils, the continuous amendment with humified exogenous organic matter leads to the improvement of SOM, as in the case of the Amazonian dark earth (*Terra Preta do Índio*, TPI) and overcomes the low crop productivity of such weathered soils [38]. The exceptional fertility of the TPI is related to the great recalcitrance of its organic matter, that shows essentially hydrophobic characteristic but it is still reactive [39].

Piccolo et al. [5] demonstrated that one of the most efficient practices for increasing the content of SOM is the amendment with composted organic matter enriched with hydrophobic groups. This assumption is based on the fact that organic matter accumulation results from restricted microbial accessibility of organic molecules and this occurs because of both adsorption and incorporation into soil mineral components, particle size-fractions and aggregates during different stages of decomposition. It is not surprising that the final quality of composted organic matter was increased with an initial addition of highly hydrophobic biochar to the raw material [40]. However, the supply of large amount of composted organic matter [or biochar] to a crop field is challenging in practice, since local territories are often deficient of suitable resources. In addition to time required to see a SOM increase and to the involved manual work, it is unreasonable to expect that food-deficient farmers spend money in buying compost. The policies of big food corporations disrupt local production systems and the smallholder farmers are continuously forced to abandon their lands to increase the workforce army to be used in urban food factories. The fact that the most serious hunger situation and food shortages are found in rural zones of peasant communities and small farmers in the north of Rio de Janeiro State is not a coincidence. Therefore, one of the major reasons for the search of plant biostimulants that would improve local crop systems is the need to decrease food insecurity and enhance biodiversity.

We observed a sharp enhancement of soil fertility with increasing distance from the homestead of smallholder farmers due to diverse allocation of resources in the different settlements at North of Rio de Janeiro state, Brazil. It is a typical soil fertility gradient found in traditional agriculture [41]. Based on this field observation, we began an alternative approach by stimulating small vermicompost productions to be used in vegetable gardens next to houses. This approach also allowed to introduce a gender-sensitive issue. In fact, most agroecology assessments focus on ecological benefits such as the refusal of chemical fertilizers and the use of crop diversification, but little attention is given to gender aspects. Vermicomposting is a typical low-cost technology for processing or converting organic waste into high-quality composts that implies the creation of conditions for women mobilization, since they are almost always responsible for the household waste and for the attention to small animals. Patriarchal heritage in the Brazilian countryside is a cultural chasm to be bridged for equality achievement, a basic Agroecology assumption.

Improving organic amendments quality may result in fast yield responses in vegetable gardens and orchards, thus enhancing food security. Besides, it encourages discussion on the role of biological inputs to overcome the problem of the transition towards inputs substitution [42]. The concept of transition to Agroecology refers to a rapid and sometimes abrupt reorganization of production models by moving to new complex systems operating within the confines of older structures. Remarkable challenges are the plant adaptation to slow-releasing of nutrients from organic sources and the sudden rise in the incidence of plant pathogens and plagues (especially ants). The use of plant biostimulation compounds may speed up cropping adaptation to the transition-phase and represent a new avenue to agroecological production models.

We conducted a campaign to provide farmers with the biological technology to produce humic extracts from vermicompost to be applied directly on the plant surface as a spray suspension. Concomitantly, we created a local research programme to understand the underlying mechanisms that positively modulate root traits, nutrient uptake and drought stress alleviation of plants treated with humic extracts. Vermicompost is a well stabilized, aesthetically pleasing, finely divided peat-like material with a refined structure, high porosity, good aeration and drainage properties, and high water-holding capacity. It has the potential to enhance plant growth [43]. In particular, the mature vermicompost is enriched with HA, which have a widely reckoned capability to induce plant development, especially for root systems [44]. The humic-like organic matter isolated from vermicomposts shows high biological activity that plays as a useful plant growth promoter [1].

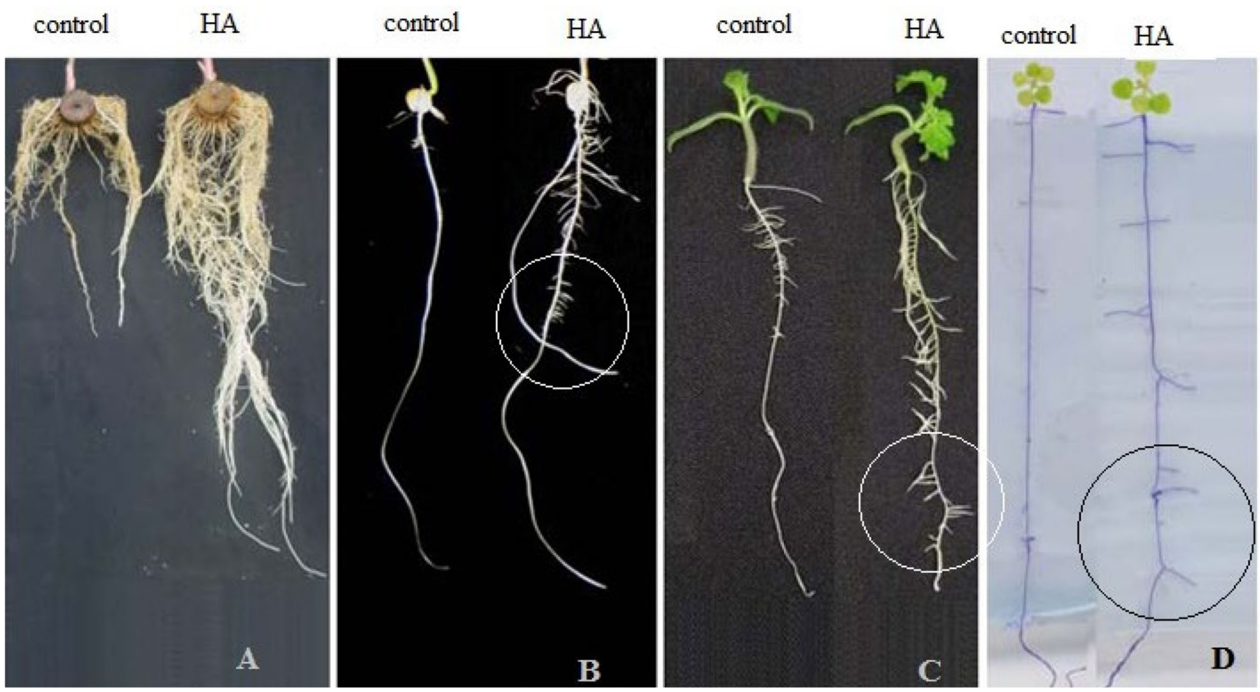


Fig. 1 Changes on root traits of different plant seedlings (**A**: sugarcane, **B**: maize, **C**: tomato and **D**: arabidopsis treated with humic acids isolated from vermicompost in comparison with control—non treated seedlings). The concentration of humic acids (HA) used varied from 4 to 5 mM C L^{-1} in the hydroponic cultivation. Note the enhancement of lateral root emergence zone (highlighted by the circles) out of the meristematic zone. (Source: personnel file of LPC)

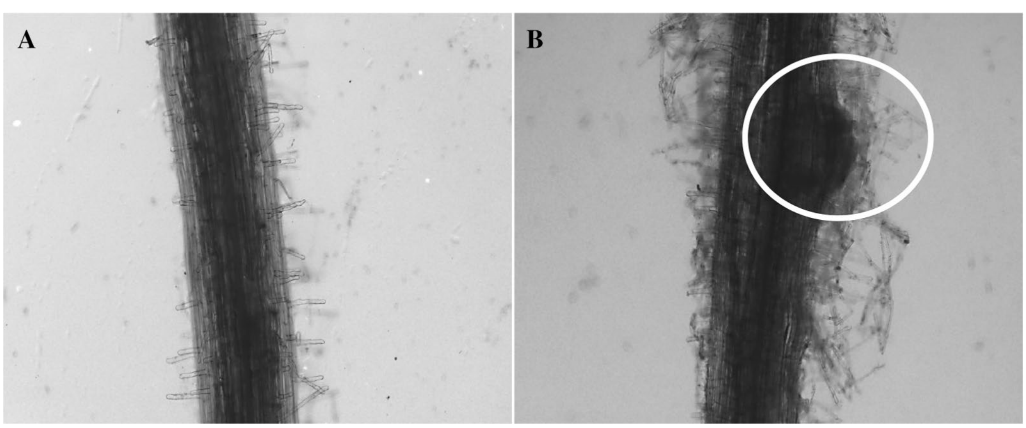


Fig. 2 Changes on root hairs pattern in maize seedlings characterized by an increased number, density and length. **A** Control, **B** maize seedlings treated for 48 h with humic acids isolated from vermicompost [4 mM C L^{-1}]. The light micrographs were obtained by FLO. The circled area in the root treated with humic acids shows a typical mitosis site for lateral root emergence. (Source: personnel file of FLO and LPC)

Root anatomy traits

One way to minimize the negative impacts of water and nutrients scarcity is to induce the root system architecture (RSA) in soil that optimizes water and nutrient uptake [45]. RSA changes are the main plant adaptation to low fertility soils and drought. Induction of lateral roots and root hair consequently increases root length and surface area [46] and enhances soil exploration and nutrient acquisition [47]. The formation and growth of lateral roots are among the most critical factors governing RSA. Lateral roots, including any subsequent higher-order branches, typically comprise the majority of root biomass and length [48].

There are several reports showing changes on root traits induced by humic matter isolated from vermicompost, including enhancement of number of lateral roots, and, consequently, the overall root surface area [49–53]. Different plants species treated with a low concentration of HA from vermicompost had shown a root phenotype characterized by increased root length and large lateral root emission zone, thus amplifying roots porous structures (Fig. 1).

Moreover, it was also observed a proliferation of number and density of root hairs in different plants species treated with HS [54, 55], including those isolated from vermicompost [56]. Root hairs ensure nutrients acquisition by expanding the physical and chemical exploitation of the rhizosphere and increasing the absorptive surface area [57] (Fig. 2).

Root physiology traits

One of the most important effects of humic substances on roots includes the nutrients uptake mediated by the synthesis and functionality of membrane proteins, related to proton pumps that increase the electrochemical proton gradient across the plasma membrane. HA can increase plasma membrane H^+-ATPase, vacuolar H^+-ATPase and pyrophosphatases activities [58], thus modulating the cellular electrical environment. H^+-ATPases couples adenosine triphosphate (ATP) hydrolysis to H^+ transport across cell membranes that result in apoplast acidification [59]. Such pH decrease activates exoenzymes to cleave the covalent links between cellulose and hemicellulose fibrils. As a consequence of such a coordinated increase of the turgor pressure, the cell walls are loosened, and the cell expansions are observed [60], thereby promoting an increase in root growth. The first report of proton pump stimulation by HS was provided by Maggioni and colleagues [61]. Due to its crucial role in ion uptake and root growth, this phenomenon has been thoroughly studied [52, 58, 62–67]. Using an ion-selective vibrating probe system, we followed in real time the ion fluxes across the rice root seedlings [68]. The emergence of the lateral root was related to specific H^+ and Ca^{2+} fluxes in the root elongation zone underlying activation of the plasma membrane H^+-ATPase and the Ca^{2+}-dependent protein kinase (CDPK). The latter was coupled with an increased expression of the voltage-dependent OsTPC1 Ca^{2+}channels and two stress-responsive CDPK isoforms, such as OsCPK7 and OsCPK17. We want to highlight these data since Ca^{2+} ions are long recognized to represent critical second messengers in many signalling pathways related to the abiotic and biotic stress response. Moreover, protein kinases also have paramount importance in cell signal transduction and are responsible for the post-translational control of target proteins and act as critical regulators of various signalling cascades [69]. Both phenomena (cytosolic Ca^{2+} pulse and induction of phosphatases) were induced by low concentration of HS. Another strong root physiological response induced by HS, but least explored, is its significant feedback effect upon the exudation process.

Root exudation is part of the rhizodeposition process, a major source of soil organic carbon released by plant roots. Inputs of labile organic solutes and sloughed-off cell tissues from plant roots can represent around 10% of net fixed C and up to 30% of the C allocated to roots [70]. This process is believed to constitute a strategy whereby growing plants improve soil nutrient availability by supplying additional energy-rich C (i.e., carbohydrates) to soil microbes capable of decomposing SOM [11, 71]. This mutual interaction between microorganisms is a crucial factor in determining soil fertility. Previous works have demonstrated that HS can significantly affect the amount of bioavailable C deposited by maize plant roots, thus resulting in a significant change in the structure of soil microbial communities [72, 73]. Puglisi et al. [74], using PCR-DGGE approach, compared the microbial diversity in the rhizosphere and the bulk soil by the Shannon index. Changes on microbial community in plants treated with HS were noticed in the rhizosphere and bulk soil 10 mm far from the rhizoplane. The significant enhancement of organic acids and sugars exudation induced by different HS were related to microbial diversity increase. The modulation of the chemical environment of the rhizosphere by HS unveiled by Puglisi and colleagues is the crucial step to understand the chemical dialogue in the plant–microorganism in order to reach a biological optimization of this relationship.

The first living frontier of complex interaction at the plant–soil interface is border cells at the root cap. Border cells (BC) are defined as cells that disperse in aqueous solutions when root tips are immersed in water [75]. BC work as external environmental cues sensors anticipating adaptive responses of plants and check the pathogenicity of the surrounding microbiota. We observed a significant increase in BC production in maize seedlings tips treated with HA [76]. An increased number of released BC induces high diazotrophic bacteria population around the root tip region, which may aggregate with mucilage and HA particles, thus enhancing their viability. Increased BC numbers in response to HA might explain previous studies showing a concomitant increase in *H. seropedicae* populations in the rhizosphere, rhizoplane, and endosphere of grasses [77, 78].

Finally, we adapted a metabolomic approach to study the level of primary (and some secondary) metabolites present in the maize root seedlings tissues and the solution trap after HA exposition [79]. Indeed, one needs to be aware of technical problems concerning the use of the solution trap methodology to evaluate exudation. However, we consider the results in relative terms, i.e., only in comparison with control treatments without additional extrapolation. The dynamics of root exudation, as well as the quality of root exudates, were not changed by HA treatment [80]. However, the yield of all exudates was amazingly increased in comparison with control plants. We confirm the previous observation that maize seedlings treated with HA enhance organic acids exudation [72, 74, 79] and other compounds such as amino acids, lipids and products of the shikimic pathway. Low mass weight compounds availability from primary and secondary metabolism promotes microbial changes at the rhizosphere, changing the plant–microorganism interaction.

Humic acids function to stress drought recovery

One of the most serious problems for small-scale agriculture is the very frequent appearance of dry periods during the normally rainy season, inducing crop yield reduction. Such uncertainty in their expectation of future economic returns leads families to discouragement. This phenomenon is known locally as *veranico* and is defined as dry days with intense heat and low relative humidity during the rainy season that negatively affects crop production. Plants previously treated with HA can counteract drought stress changing physiological and biochemical processes, including accumulation of compatible solutes and activation of detoxification enzymes [81]. The authors first submitted sugarcane seedlings to an HA treatment. Then the irrigation water was cut off to induce drought. When the relative water content reached a critical point in the control, the irrigation was resumed, and the seedlings were evaluated. It was observed that after rehydration the activity of antioxidant enzymes catalase, superoxide dismutase, peroxide (CAT, SOD and APX) remained larger in leaf and root tissues of HA-treated plants than in control. It was also observed that HA changed the metabolic profile of rehydrated sugarcane leaves after the drought step. ^{1}H NMR spectra highlighted the enhancement of aromatics, sugars, methoxyl and aliphatic compounds [mainly fatty acids] in response to humic treatment. Changes in the metabolic profile of sugarcane treated with HA were also observed without drought stress showing a general metabolic boost induced by HA [82]. The seminal work of Nardi et al. [83] had previously shown that HA could enhance the activity of key enzymes of the glycolytic and tricarboxylic acid pathway in maize seedlings. Before finishing this introductory part and presenting the case study, we will briefly address two more aspects related to the limitation of nitrogen and phosphorus in highly weathered soils.

Low phosphorus and nitrogen concentration

Crop production in smallholder systems is limited by multiple agronomic and pedoclimatic factors that operate simultaneously and cause resource imbalances that eventually lead to poor yields [84]. To summarize this set of problems as a soil nutritional deficiency is a rough oversimplification. However, it is a fact that in highly weathered soils, low levels of available nitrogen (N) and phosphorus (P) can severely affect crop yield.

Despite the large amounts of total P in tropical acidic soils, where Al and Fe are dominant, the bioavailable forms of P are minimal, due to (1) inorganic phosphate (Pi) retention by oxides and clay minerals, (2) and P immobilization into organic forms [85]. Plants develop adjustable mechanisms to low available P such as optimization of root biochemistry to acquire soil P in rhizosphere through increased gene expression and activity of Pi transporters, the secretion of acid phosphatases as well as organic acids to release P from the soil, and the optimization of internal P use [86].

Primary or secondary minerals with N are rare in the tropical environment, and the main N forms are present in organic compounds that must be first mineralized and then released as either ammonium or nitrate ions. The continuous soil organic amendment and litter turnover can provide N and P in suitable quantities to support crop production in systems under equilibria between mineralization and immobilization process. The ebullience and biodiversity of tropical forests are clear examples of these balanced turnovers. The problem arises when the natural vegetation is removed, burned, and replaced with monoculture settles for years on end.

The most of N is present in the atmosphere as dinitrogen (N_2) that can be naturally fixed and converted to NH_3 by different microorganism species in a process termed biological nitrogen fixation (BNF). Plants have developed different evolution strategies to supply carbon compounds to these microorganisms in exchange for atmospheric N. The ecological relevance and economic impact of BNF can be scaled by the Bradyrhizobia and soybean symbiosis in Brazil. In non-leguminous plants, diazotrophic bacteria also provide magnificent contributions. For example, sugarcane had evolved an efficient association with N_2 fixing bacteria capable of fixing large amounts of N in the range of 100 kg $N.ha^{-1}$ and more [87]. As shown previously, HS can induce modifications on soil microbiota, and, when directly applied to plant surface, can enhance the number of endophytic diazotrophic bacteria in shoot and root tissues resulting in crop yield increase [77].

Both P and N are present in very low concentration in the soil solution of highly weathered soils, and both are preferentially absorbed by plants in the anionic species (NO_3^-) and ($H_2PO_3^-$, HPO_3^{2-}, Pi). Both NO_3^- and Pi uptakes proceed against the electrochemical gradient at the plasm membrane and require active transport energized by plasma membrane H^+-ATPase activity. The NO_3^- uptake is a 2:1 symport ($2H^+$: 1 NO_3^-) while Pi is a 1:1 symport system. Two groups of transporters co-occur in the plasma membrane of root cells and act in a coordinated way to absorb NO_3^- and Pi over a wide concentration range. At high N and P concentration, the low-affinity transport systems (LATS) is active, and at the low concentration, the high-affinity transport system (HATS) is induced. The HATS includes NRT2.1 and NRT2.2 for NO_3^- and PHT1 and PHT2 for Pi. These components of NO_3^- and Pi uptake (LATS and HATS) are directly energized by plasma membrane H^+-ATPases and need to be coordinated with the availability of NO_3^- or Pi in the medium for more efficient absorption. If there is a certain consensus about the positive effect of HA stimulation on plasma membrane H^+-ATPases activity, its impact on HATS transporters is not simple. For example, it was previously reported that HATS of NO_3^- (ZmNRT2.1 and ZmNRT2.2) were not induced by HA exposition in maize roots, while in shoots an increase in transcription level was observed [67]. The transcription level of HATS on roots of rice seedlings (OsNRT2.1 and OsNRT2.2) was three times larger than that of control after 48 h of exposure to HA [88]. These authors also found modifications on traditional Michaelis–Menten kinetics parameters by HA such as an increase in the V_{max} and reduction of the K_m and C_{min} values for NO_3^-, thus facilitating its uptake at low concentrations. However, it was also detected a repressed transcription of genes ZmNTR1 and ZmNRT2 in maize roots seedlings treated with HA isolated from vermicompost [89].

The influence of HA on Pi transporters is even less studied, but it has been pointed out a positive stimulation of the transcription level of HATS of Pi (LePHT1 and LePHT2) in root seedlings of tomatoes treated with HA at low Pi concentrations [36]. However, the LePHT2 was also found in greater transcription levels than control with high Pi concentration, showing that the nutrient perception at the rhizosphere can be disturbed by the HA treatment [36]. The perception by roots of the external concentration of Pi and other nutrients is a complex event and it is hard to uncover the mechanisms using our fragmentary system of nature description. However, despite the persistence of complexity, it is more convenient to monitor the processes inside the plants.

The coupling of available nutrients and energy with growth factors is required for cell proliferation [90]. The primary function of the target of rapamycin (TOR) kinase is to promote cell growth in response to favourable conditions of nutrients and energy, acting as cell sensor and signalling pathways regulating the perception and the reactions to nutrients (sugars and amino acids) and energy levels [90]. In other words, when sugars and amino acids concentrations are at an adequate level, the TOR complex is activated. We monitor the sugars, organic acids, amino acids and lipids content in root and shoot tissues using gas chromatography coupled to a time-of-flight mass spectrometry (GC-TOF/MS) and differential transcriptional level of TOR in maize seedlings treated or not with HS isolated from vermicompost [91]. The results were not as expected since the transcription level of TOR was larger in treated plants despite the low content of sugars and amino acids in plant tissues. The activity of the TOR complex can be activated without enhancing gene expression. However, it is also apparent that HA somehow modify the cell perception of nutrient and energy status, de-coupling the plant level of nutrients and TOR expression. It is not easy to extrapolate these results out of the controlled conditions. However, it is possible to speculate that HA may hinder the perception of nutrient concentrations, thus allowing the plant to develop its growth even under nutrient-deficient condition, typically found in highly weathered soils.

Considering all of these results and effects of HA isolated from vermicompost on root morphology and physiology, we directed the attention to both the time needed by vermicomposting to obtain bioactive HA and a putative relationship between the composition of organic residues used in the composting process, as well as the structural features of HA isolated from and their bioactivity. We started producing vermicompost using different organic residues as sugarcane bagasse rich in lignins, sunflower cake rich in lipids, and filter cake of sugarcane factory rich in carbohydrates mixed (3:1/*v*:*v*) with cattle manure. The vermicompost HA were isolated at different vermicomposting times, and their bioactivity was measured by the number of lateral roots and H^+-ATPase activity. Results showed that HA were bioactive after 45 vermicomposting days, and showed a clear relationship between structure and bioactivity [40]. Using chemometrics tools it was was possible to predict the bioactivity of HA, considering the number of lateral roots and H^+-ATPase activity [92]. The nuclear magnetic resonance spectroscopy in the solid-state (^{13}C-CPMAS-NMR) and diffuse reflectance Fourier transformed (DRIFT) infrared spectroscopy were used to analyse the vermicompost and HA composition. The main variables (higher loadings) correlated with HA bioactivity were for NMR positive loadings for lignin (56 ppm, 124 ppm, 148 and 153 ppm) and COOH groups (174 ppm), and, in DRIFT spectra, aryl stretching and bending signals (1560, 1480, 860 and 780 cm^{-1}) probably from lignins, and the C–H asymmetric and symmetric stretching bands at 2926 cm^{-1} and 2852 cm^{-1}, respectively, in long-chain fatty acids. The model was tested using HA artefacts from cattle manure vermicompost produced by different chemical reactions (oxidation, reduction, methylation) and partial removal of functional groups by solvents [93]. Again, it was possible to observe a clear relationship about chemical structure and bioactivity, showing that the hydrophobicity index was significantly related to the proton pump stimulation. It is suggested that the hydrophobic domain can preserve bioactive molecules such as auxins encapsulated in the humic matrix. In contact with root-exuded organic acids, the weak hydrophobic forces could be disrupted, releasing bioactive compounds from the humic supramolecular association. These findings were further supported by the fact that all used derivatives enabled activation of the auxin synthetic reporter DR5::GUS. The potential release of bioactive molecules from the humic supramolecular associations in the rhizosphere system confers the plant biostimulation capacity. In the rhizosphere environments, the low aqueous solubility and the considerably enormous surface tension triggered by the hydrophobic humic components foster the interaction with the root systems. In the rhizoplane, the large concentration of root exudates (inorganic ions, organic acids, siderophores, amino acids, etc.) increases the physical–chemical interactions with the weakly bound supramolecular structures [94]. The dynamic and flexible humic conformation may then undergo a favourable thermodynamic rearrangement with the subsequent possible release of retained polar bioavailable molecules which can exert the bioactive properties in the proximity of root membranes. The overall hydrophobic character and the concomitant presence of easily releasable bioactive components such as oxidized lignin derivatives and polar O-alkyl and nitrogenated moieties confer great bioactivity to HA isolated from vermicompost.

HS and HA are traditionally considered as recalcitrant natural organic matter. Few microorganisms can use HA as a source of energy. As we have already seen, HS modify the plant exudation and the bacteria communities. HA are applied in our study as a vehicle of beneficial bacteria delivery to the crop field. We start using annual crops (maize, tomato, and common beans) and sugarcane. Some related details are found in a review of our previous works [95]. However, the use of soluble humic matter whether in combination or not with perennial cultures is scarce. Perennial agroecological systems have the potential to overcome some problems of small farmers discussed previously, including a decrease of handwork intensity and reduce the need for external inputs and hence provide a viable alternative for farmers.

We report here the case study where we used the application of humic-like fractions isolated from cattle manure vermicompost and endophytic diazotrophic bacteria (*H. seropedicae*) in different tropical fruits, including banana, passion fruit, papaya and pineapple in an on-farm experiment. The following sections comprise a brief description of how the experiments were conducted.

Materials and methods

Experimental site

The on-farm experiment (0.5 ha) was installed in the small farm (7.0 ha) located at Campos dos Goytacazes, north of Rio de Janeiro State, Brazil (21° 45′ 16″ S, 41° 19′ 28″ W), and surrounded by large cattle ranching farms. According to oral reports from elder local people, the land property was originally derived from an asset of the ancient large sugarcane family that had been successively distributed among family members, and some of these parts have been sold. Sugarcane plantation was removed more than 30 years ago, and the native grass (*Paspalum mandiocanum*) was established. Regarding landscape, the relief is in the range of smoothly wavy to wavy, typically made up of small hills with half an orange shape interspersed with small valleys with a predominance of Ultisols and Alfisols in the most prone area and fluvisols in the plain. The climate is A_w according to the Koppen classification system, i.e., humid tropical climate with rainy summer and warm and dry winter.

Humic acids and plant-growth promoting bacteria

Soluble HS were extracted from vermicomposted cattle manure with 0.1 M KOH at a 1:20 solid–liquid ratio by mechanical shaking for 6 h. The suspension was centrifuged at 5000*g* and acidified until pH 2.0 with 6 M HCl. The pellets of the resulting HA were resuspended in the extracting solution, acidified to pH 7 with 6 M HCl, and dialyzed against deionized water (Spectrapore 000-D cutoff membrane). The HA solution was finally freeze-dried before further use. The total carbon and nitrogen content were determined using a CHN Perkin Elmer autoanalyzer (Perkin Elmer series 2400, Norwalk, CT, USA). The molecular characteristics of HS were determined by ^{13}C-CPMAS-NMR spectroscopy recorded on a Bruker AV-300 (Bruker, Karlsruhe, Germany) equipped with a 4 mm wide-bore MAS probe, with the following acquisition parameters: 10,000 Hz of rotor spin rate, 2 s of recycling time, ^{1}H-power for CP 92.16 W: 1H 90° pulse 2.85 μs, ^{13}C power for CP 150, 4 W, 1 ms of contact time, 30 ms of acquisition time, 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps.

The Free Induction Decay (FID) was transformed by applying a 4 k zero filling and an exponential filter function with a line broadening of 150 Hz. The overall chemical shift range of ^{13}C-CPMAS-NMR spectra was split into six regions assigned to the main organic functional groups (22, 23): 0–45 ppm (aliphatic-C), 45–60 ppm (methoxyl-C and N-alkyl-C), 60–110 ppm (O-alkyl-C), 110–145 ppm (aromatic-C), 145–160 ppm [O-aryl-C], 160–190 ppm (carboxyl-C). The relative contribution of each carbon group was estimated by dividing the area of the corresponding spectral interval by the total spectral area $\times$ 100.

Herbaspirillum seropedicae strain Z67 was kindly provided by Dra Verônica Reis from Embrapa Agrobiologia and grown in vials with a complete JNFb semisolid medium. The composition of JNFb-medium per litre is: malic acid (5.0 g), K_2HPO_4 (0.6 g), KH_2PO_4 (1.8 g) $MgSO_4{\cdot}7H_2O$ (0.2 g), NaCl (0.1 g), $CaCl_2$ (0.02 g), 0.5% bromothymol blue in 0.2 N KOH (2 mL), vitamin solution (1 mL), micronutrient solution (2 mL), 1.64% Fe•EDTA solution (4 mL), KOH (4.5 g). In 100 mL, the vitamin solution contained: biotin (10 mg) and pyridoxal-HCl (20 mg), 1 L of the micronutrient solution consisted of: $CuSO_4$ (0.4 g), $ZnSO_4{\cdot}7H_2O$ (0.12 g), H_3BO_3 (1.4 g), $Na_2MoO_4{\cdot}2H_2O$ (1.0 g) $MnSO_4{\cdot}H_2O$ (1.5 g). The pH was adjusted to 5.8, and 1.9 gL^{-1} of agar was added, and the bacteria were grown at 34 °C for 16 h with shaking (150 rpm). Cells were pelleted by centrifugation (4,000 g for 15 min) and resuspended in sterilized water at cell densities of 109 colony-forming units (CFU) mL^{-1}.

The treatment consists of a HA suspension (2 mM C) and *H. seropedicae* (10^9 cel mL^{-1}) administered by dripping after each specie field transplanting. The inoculant was prepared to dilute 200 mL of bacterial in 800 mL of HA at pH 5.8 ± 0.5 to produce a final concentration of 48 mg C per litre (2 mM C) and a final bacteria concentration 5×10^8 cells/mL. The manual spray of suspension was applied on the leave surface until the dripping point equivalent to approximately 400 to 450 L per ha.

Fig. 3 **A** Soil tillage by oxcart in pineapple and papaya plantation that succeed banana plantation; **B** general view of plant layout for banana, pineapple and papaya

Experiment layout

The on-farm experiment carried out in farmers fields at the foothills at yellow Alfisol whose chemical characteristics were shown in sample 2 of Table 1. The implementation was initiated with plantation of the four lines of banana seedlings (*Musa paradisiaca*, cv. *Maravilha*) with the width of 6 m between 2 lines and 3 m between seedlings. The pits were fertilized with 20 L of cattle manure before plantation. The plantation was conducted in September, and the suspension containing the inoculant and HA was applied twice (five days and 30 days after plantation). We applied to one plant, the volume equivalent to 450 L ha^{-1}. The treatments were arranged in randomized blocks with four repetitions. During the summer of the first year of banana plantation occurred a period of intense heat and drought during the rainy season, and

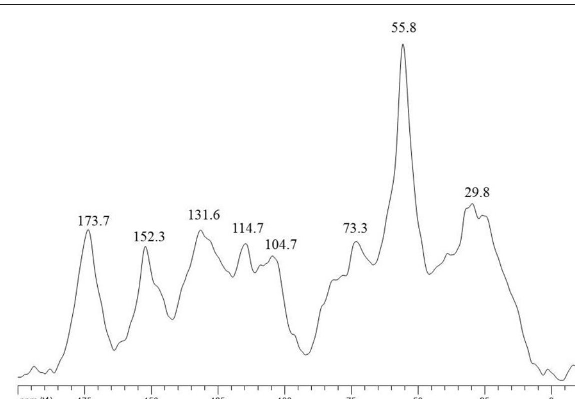


Fig. 4 CP/MAS ^{13}C NMR spectrum of humic acids

the seedlings were manually watered once a week during January with a 20-L bucket of water per seedling.

A line of papaya (*Carica papaya* var. UENF/Calimã) was set up at 2 m of each side of banana lines with 2 m between plants. At parallel, a double line of pineapple (*Ananas comosus* var. pérola), with 1 m between lines and 0.5 between plants, was placed between banana and papaya line. All lines were first tilled by the oxcart plough and the opening of pits (0.3 × 0.3 m) was performed manually. All pits destined to papaya plantation received 20 L of cattle manure before the seedling transplant and 10 L from pineapple plantation. During the cropping season, manual weeding was carried out on the plant lines, and between the lines. The native grass was maintained and cut with a gasoline manual mower when necessary and the residues left on the ground. After the first year, the pineapple lines were covered with 10 cm of mulching obtained with the cut of native grass from a nearby experiment and manually displayed to reduce the velocity of weed growth in the raining season. The treatments were arranged in four completely randomized blocks with four banana trees, six papaya trees and 12 pineapples per repetition. In case of the passion fruit experiment, we consider the experiment in strip with three lines of each treatment. The fruits were harvested and only weighed. No additional measurement was done (Fig. 3).

Six lines of passion fruit (*Passiflora edulis* cv. vermelho) with 3.5 m between lines and 3.0 m between plants were settled next to the last line of banana towards the hill. Pits of 0.4 × 0.4 m were manually opened and fertilized with 20 L of cattle manure before the red fruit of passion plantation. The plantation was done in September with a trellis driving system. Five days after plantation, the inoculant was applied as a foliar spray and reapplied after 30 days on tree alternated lines. The seedlings were pruned when hit the supporting wire. The weeds control was done manually around the seedlings, and 150 mL of vermicompost was added twice a year (October and February). The caterpillar incidence was manually controlled.

The seedlings were conventionally produced at Embrapa Agrobiologia (Seropédica, Brazil), except for papaya produced at UENF (Campos dos Goytacazes, Brazil). No other types of agrochemicals and irrigation were used. The pineapple flowering was not induced.

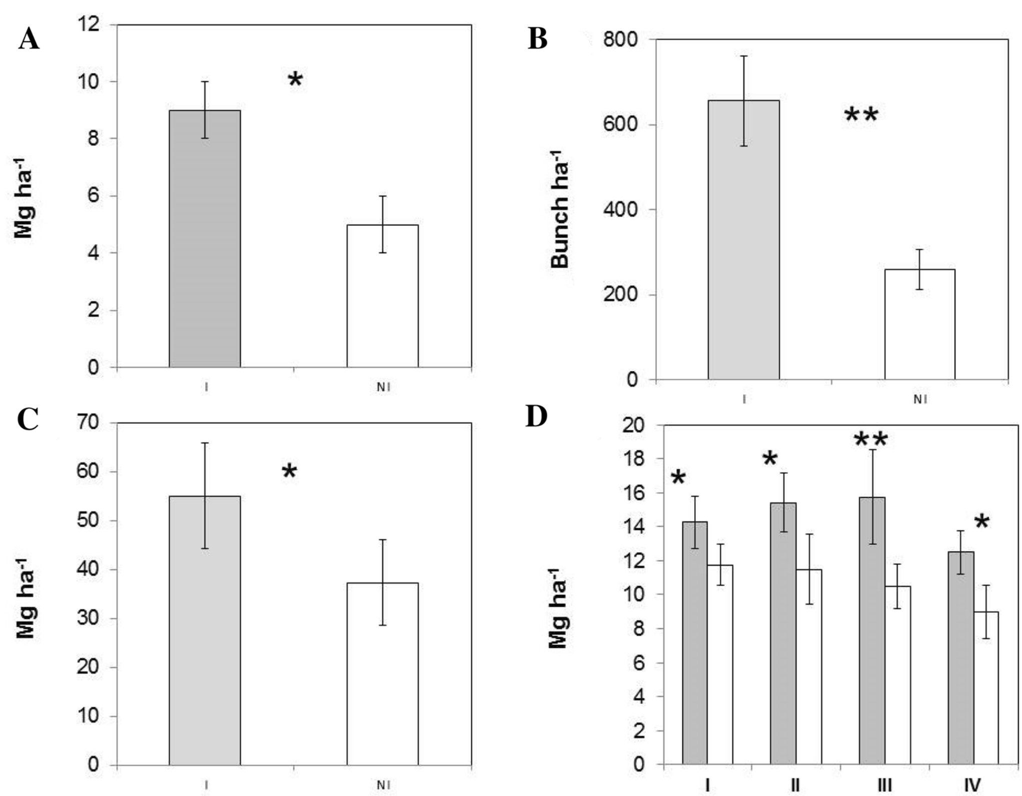


Fig. 5 Effect of application of humic acids (HA) and plant-growth promoting rhizobacteria (PGPR) on banana growth: **A** fruit yield (Mg) per area (ha). **B** Number of banana bunch per area (ha). **C** Papaya yield. **D** Red fruit of passion yield during our consecutive harvest. Gray bars and white bars represent the treatment with HA + PGPB and white bars the control without inoculation. The bars represent the stand error and * and ** is the significance of *F* test of $p < 0.01$ and $p < 0.05$, respectively

Results and discussion

The ^{13}CCP/MAS NMR spectrum of humic acids used in the biostimulant preparation is shown in Fig. 4. The presence of alkyl-C groups at 0–45 ppm is associated to the presence of aliphatic chains (CH_3–$CH_{2(n)}$). The sharp signal centred at 55.8 ppm combine either the methoxyl substituent on the aromatic rings of lignin components, as well as the C–N bonds in amino acid moieties. The shoulder at 73 ppm is formed by the overlapping resonances of carbohydrates and the resonances at 104.7 ppm is due the presence of anomeric carbon. The broad bands extended along the aryl-C interval (114–131.6 ppm) involve the un-substituted of different aromatic components, while the signals at 152.3 ppm are due the presence of phenolic compounds (152.3 ppm). Finally, the sharp signal at 173.7 ppm includes all carbonyl and carboxyl groups. The content of organic carbon and total nitrogen was 43% and 3.2%, respectively, after ash determination.

The inoculation with HA and PGPB increased the banana yield expressed in ton ha^{-1} (Fig. 5a). The number of bunch per ha was also larger in treated plants (Fig. 5b). In treated plants, the production was anticipated in both the first (350 vs. 410 days from transplanting to harvest) and second (600 vs. 660 days) year of production. However, the most extraordinary results were related to the significant decrease in the mortality rate of seedlings after planting when a severe drought occurred. While in the no-inoculated seedlings, the mortality rate was around 40%, only 5% of inoculated seedlings were lost after transplanting.

The papaya production was also increased by inoculation, including total yield (Fig. 5c) and number of fruits per plant. The fruit weight did not change with treatment, including the number of commercial fruits (as by 800 g per fruit) that remained the same both for control and treated plants.

The pineapple yield was low (15 ton ha^{-1}) in comparison to conventional monoculture pineapple yield, as obtained at the north of Rio de Janeiro State (around 30 ton ha^{-1}), and was therefore not modified by the inoculation (data not shown). However, it was observed a wide variation in the time of fructification since the flowering was not induced artificially. This would represent a profitable strategy since mature fruits were practically observed all year after 18 months from plantation. It was not detected any difference among number, weight or size in pineapple fruits, while the number of slips per plant increased significantly from 4 ± 1 in control plants to 11 ± 1 in treated plants. The pineapple seedlings from local and adapted slips allowed to expand the crop production in the following consecutive year without expending time or resources to obtain the seedlings.

The crop yield of the red passion fruit was larger in treated plants (Fig. 4d). The decrease in fruit yield over the production cycles was smaller in the treated plants than in control. No significant change was shown in fruit size and weight due to a larger number of flowering emergences and fruits per plant.

The results obtained with the application of HA and PGPB by foliar spray on crop yield accredited the little environmental impact of the employed biotechnology, as an important tool in the transition process. We do not emphasize the results in crop yield in the context of the current overexploiting productive model, but we underline the advantage in terms of an alternative crop system considering the perennial polyculture towards a substitution of industrial chemical inputs. There are three important issues in the transition process to a more sustainable agriculture: (1) rationalization measures, (2) inputs substitution, and, (3) redesign the agroecosystems [96]. During the transition, replacing expensive and the excess amount of industrial chemical inputs by alternatives, sometimes cheaper, must not be neglected. Biological or alternative inputs are also evident, although inputs substitution does not necessarily lead to more sustainable agriculture since farmers dependency can remain the same [37]. More than twenty years ago, these authors wrote as follows: "*The input substitution approach only emphasizes environmentally benign alternatives to agrochemical inputs, without challenging either the monoculture structure or the dependence on off-farm inputs that characterize agricultural systems*" [37], that is in line with our opinion. The risk of appropriation of biological inputs by modern industrial agriculture is real, as it is also the risk of agroecology being institutionalized, disconnected from social movements and transformed in ecological practices, or worse, in scientific papers in a technical language inaccessible to farmers. Furthermore, we reinforce the need to know how the biological inputs work and what are their field limitations when they coincide with the end of external dependence on their production. Unlike chemical inputs that have defined molecules and active principles and defined targets for action, biologicals inputs have a more complex relationship and depend on interaction with ecological processes.

Within this context, it is important to report alternative inputs and technologies that gradually contribute to the re-establishment of ecological processes, which can reduce the external dependence on chemical inputs, thus contributing to limit the use of polluting products with their notorious environmental hazards and energy impacts, and to produce beneficial social results [97]. These authors also pointed out that soil is of central concern in the transitional process because its adequate recovery relies on obtaining continuous productivity indexes over time without employing large doses of chemical fertilizers. The incorporation of functional agricultural biodiversity and the use of biostimulants represent a technological advance in the substitution of industrial inputs.

The increased use of commercial humic products had remarkably emerged in the last years based on indicators such as the number of end-users, applied field size, and market expansion. In Brazil, more than one hundred companies sell a range of commercial products, sometimes at extraordinarily high prices. We will not discuss the effectiveness of these commercial products, but we must notice that slick marketing from companies tries to convey the idea that commercial HS-based biostimulants are eco-friendly products, while the primary sources of such humic extracts are non-renewable lignite/leonardite and peats ores, whose exploitation endangers the ecosystems.

Vermicompost has been suggested as a substitute for peat in substrates [98] and has, in its constitution, large concentrations of highly bioactive HA [99]. The HA extraction from vermicompost with alkali solutions is a "handy" application procedure, and even farmers with low-education background may apply such an easy extraction method. Most commercial humic products are in the market as soil conditioners, and recommendations of use refer to small doses (10–50 kg ha^{-1}) in a solid formulation directly applied to the soil. The poor effectiveness of such use has already been underlined [100]. Conversely, we postulate the use of soluble humic solutions at low concentration directly on the surface of plant leaves. The downside benefit of the proposed technology of the combined use of HS and bacteria is the maintenance of beneficial strains selected in the laboratory, keeping them active for future field utilization. However, we do not have up to now a solution for the issue of practical use of combined microbes-HS products. Although it is relatively easy to maintain the collection with commercially available growth medium, it requires a minimum degree of specialization for their handling in the files that only cooperatives or associations of smallholder farmers can have. However, the use of beneficial bacteria is essential in the reported transition process.

PGPBs are a diverse group of bacteria capable of promoting growth and yield of many crops as a result of several effects on the host, including a wide variety of mechanisms such biological nitrogen fixation, phosphate solubilization, alleviation of abiotic stress, siderophores production, rhizosphere engineering, production of 1-aminocyclopropane-1-carboxylate deaminase (ACC), quorum sensing (QS) signal interference and inhibition of biofilm formation, phytohormone production, exhibiting antifungal activity,

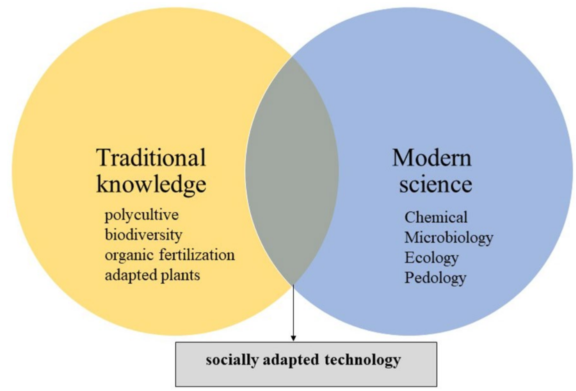


Fig. 6 Summary scheme showing the interaction of traditional wisdom and advanced science to generation of socially adapted technology for sustainable agriculture

induction of systemic resistance, promoting beneficial plant–microbe symbioses and interference with pathogen toxin production [95]. The existence of a farming system legacy influencing the biotic interactions among solubilizing phosphorus bacteria was previously demonstrated [9, 101]. The output of the interactions (cooperation or antagonism) was not phylogenetically determined. The beneficial bacteria community can be restored in sites conducted with organic or agroecological practices, but in the first step of a transition level is important to introduce efficient strain to BNF or other desired ecological target process. The introduction of selected strains means accelerating production until the re-establishment of microbial diversity and the intensification of natural ecological processes.

A key point for restarting the crop production in areas degraded by abandoned monoculture is the introduction of fruit trees. They contribute to the soil protection due to large carbon inputs into the soil by roots and by litter decomposition. The biodiversity obtained with the intercropping of species also contributes to two important but sometimes overlooked aspects: diversity of foods for a healthier diet and significant changes in the landscape and the work environment. The shade of the intercropping of tropical fruit brings more comfort to work. The diversity of fruits forces a change in the diet. It pushes the farmer into the short circuits of direct marketing, presenting an alternative route to the usual brokers of monoculture products. According to Do et al. [102], agroforestry systems were expected, in the long term, to achieve greater net present values than monocultures. However, despite the relatively low economic return, monocultures are preferred by farmers. The simulations model reveals a long time lag before the initial agroforestry investments pay off [102]. This leads to lower short-term profits in agroforestry compared to monocultures. Resource-poor farmers often need to prioritize their families' immediate needs and do not have the luxury of being able to make income sacrifices in the short term [103]. The use of biostimulants can promote the acceleration of transition process enhancing the crop yield in the short time. This can be an important factor in changing the crop designs. Another important issue is the experience in Vietnam from farmer-driven cooperatives of small-scale organic producers that propose potential solutions to address some of the challenges to agroforestry adoption [102]. Cooperatives, with clear leadership structures and predictable commitments by a number of farmers, can offer opportunities to overcome the problem of PGPB production.

The introduction of other agricultural and forest species is the next step to increase system complexity. The beginning of the transition was based on introducing fruit trees aiming to generate income besides improving the environment rather than relying on horticultural species that need greater intensive care. The use of biostimulants promoted a significant increase in production and, when used in the proper context, can serve in the transition process by modifying social and ecological aspects in addition to the productive ones.

Conclusion

Soils threats due to modern industrial agriculture (accelerated erosion, desertification, salinization, acidification, compaction, biodiversity loss, nutrient depletion) put our civilization at stake. However, the loss of SOM is what most affects the transition to an ecology-based agriculture to be faced by small and poor farmers in the tropical zone dominated by highly weathered soils. We presented here a case study involving the use of humus-based biostimulants applied on intercropping of tropical fruits (banana, papaya, pineapple, red passion fruit). It is widely recognized that soil organic residues amendment can improve the content of SOM and restore the soil functions. We cite here some excerpts from the seminal work of Waksman [104], one of the pioneers in the study of humus in a biological perspective: *The importance of humus in the soil is manifold: it serves as a source of nutrients for plant growth, it modifies the physical and chemical nature of the soil in various ways, it regulates and determines the nature of the microbial population and its activities, by supplying sources of energy and various organic and inorganic nutrients essential for their growth, and by making the soil a more favourable substrate for their development. One should consider further the colloidal effects of humus on the soil, its buffering properties which modify the soil reaction, its combining power with bases, its influence upon the oxidation–reduction potential of the soil, its adsorption of certain toxic materials injurious to plant growth, its ability to supply certain agents and small quantities of certain rare elements essential for plant growth, its influence upon soil structure, upon the moisture-holding capacity of the soil, and soil temperature, as well as numerous other reactions which are of direct or indirect importance to plant growth.*

However, to increase SOM is a major challenge in monocultural production of highly weathered soils. Waksman also indicated the role of plant-growing substances in humus and the possibility to use humus as a booster of plant nutrition. We evaluated the effects of humic-like substances isolated from vermicompost on root traits related to more soil exploration, and we applied them in combination of PGPB after field transplanting. The crop yield was increased, and the landscape was changed. Once placed within a political and social context, it is possible to indicate that humus-based biostimulants can increase the chances of success in a transition process to agroecological practices (Fig. 6).

Abbreviations

HS: Humic substances; HA: Humic acids; PGPB: Plant-growth promoting bacteria; GC-TOF/MS: Time-of-flight mass spectrometry; LATS: Low-affinity transport systems; HATS: High-affinity transport system; BNF: Biological nitrogen fixation; Pi: Inorganic phosphate; CAT: Catalase; SOD: Superoxide dismutase; APX: Peroxide; NMR: Nuclear magnetic resonance; BC: Border cells; PCR: Polymerase reaction chain; DGGE: Denaturing gradient gel electrophoresis; CDPK: Ca^{2+}-dependent protein kinase; ATP: Adenosine triphosphate; RSA: Root system architecture; SOM: Soil organic matter; TPI: *Terra Preta do Índio*; MST: Social movement of landless workers; Nudiba: *Núcleo de Desenvolvimento de Insumos Biológicos para Agricultura* Or Center for Biological Inputs Development; ^{13}C-CPMAS-NMR: Nuclear magnetic resonance spectroscopy in the solid-state; DRIFT: Infrared with diffuse reflectance and Fourier transformed; FID: Free induction decay; ACC: 1-Aminocyclopropane-1-carboxylate deaminase; QS: Quorum sensing.

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Author contributions

LPC, FLO conceived the concept and write the first version. NOA did the chemical and statistical analysis. RCCR and JK contributed to field experiments. AP discussed the concepts and provided the final version of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate
This manuscript is an original paper and has not been published in other journals. The authors agreed to keep the copyright rule.

Consent for publication
The authors agreed to the publication of the manuscript in this journal.

Competing interests
The authors declare that they have no competing interests.

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