The contribution of Stylosanthes guianensis to the nitrogen cycle in a low input legume-rice rotation under conservation agriculture

Journal Article

Author(s): Zemek, Oliver; Frossard, Emmanuel; Scopel, Eric; Oberson, C.

Publication date: 2018-04

Permanent link: https://doi.org/10.3929/ethz-b-000263249

Rights / license: In Copyright - Non-Commercial Use Permitted

Originally published in: Plant and Soil 425(1), https://doi.org/10.1007/s11104-018-3602-0 **REGULAR ARTICLE**



The contribution of *Stylosanthes guianensis* to the nitrogen cycle in a low input legume-rice rotation under conservation agriculture

O. Zemek D · E. Frossard · E. Scopel · A. Oberson

Received: 26 April 2017 / Accepted: 12 February 2018 / Published online: 24 February 2018 © Springer International Publishing AG, part of Springer Nature 2018

Abstract

Background and aims Legumes integrated in crop rotations are intended to improve crop nitrogen (N) supply and yield. In conservation agriculture (CA) systems under low input conditions on highly weathered tropical soils, experimental evidence for these benefits is lacking. To understand the mechanisms and evaluate the impact of the legume N on the subsequent crop, an in-depth study on N dynamics in the soil-plant system was conducted. Methods In Madagascar, a CA based crop rotation with the perennial forage legume Stylosanthes guianensis (stylo) and upland rice (rice/stylo - stylo - rice/stylo) was established under three fertilization regimes. In addition, rice was grown in a non-CA bare fallow rotation without fertilizer. Over the three years N₂ fixed in stylo shoots, the incorporation of stylo shoot (mulch) N into soil N pools and mulch N uptake by rice was quantified using ¹⁵N techniques and mulch and stylo root residue decomposition was investigated in a litterbag study.

Responsible Editor: John A. Kirkegaard.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-018-3602-0) contains supplementary material, which is available to authorized users.

O. Zemek (⊠) · E. Frossard · A. Oberson Institute of Agricultural Sciences, ETH Zurich, Eschikon 33, 8315 Lindau, Switzerland e-mail: oliver.zemek@posteo.net

E. Scopel

UPR AÏDA, CIRAD, Avenue Agropolis, 34398 Montpellier cedex 5, France

Results N_2 fixed in stylo shoots ranged from 96 to 122 kg N ha⁻¹. Between 50 to 70% of stylo mulch and root residues decomposed during the third cropping season. Without fertilizer, grain yield of rice after the fallow with stylo was about 70% greater than after bare fallow, corresponding to 11 kg N ha⁻¹ greater N uptake. Recoveries of stylo mulch N after rice harvest were on average 64% in soil, with about 3% in each of the microbial and mineral N pools, with 39% on the soil surface, and 6% in the rice crop. The N input via stylo seed, leaf litter and belowground N totalled about three times the amount of N contained in stylo mulch, which usually is considered as major rice N source.

Conclusions Legumes, like stylo, can improve crop N supply and yield in low input CA cropping systems on highly weathered tropical soils. To explain the impact and mechanisms involved requires a consideration of all legume-N components beyond the mulch N present at the onset of the rice-cropping season.

 $\begin{array}{lll} \mbox{Keywords} & {}^{15}\mbox{N} \cdot \mbox{Mulch} \cdot \mbox{Symbiotic} & N_2 \mbox{ fixation} \cdot \mbox{Madagascar} \cdot \mbox{Litterbag study} \cdot \mbox{Ferralsol} \cdot \mbox{N} \mbox{ recovery} \end{array}$

Abbreviations

Conservation agriculture
CA based upland rice - stylo fallow rota-
tion were rice received no fertilizer
CA based upland rice - stylo fallow rota-
tion were rice received farmyard manure
CA based upland rice - stylo fallow rota-
tion were rice received mineral fertilizer

DM	Dry matter
MC-NON	Mono cropped upland rice - bare fallow
	rotation were rice received no fertilizer
%Ndfa	Proportion of total N derived from the
	fixation of atmospheric N ₂
%Ndfm	Proportion of N derived from stylo mulch
	N in a plant or soil N pool
SD	Standard deviation

Introduction

A sustainable yield increase on highly weathered soils in low input cropping systems of the tropics requires management practices that maximize the use of available resources, control pest and diseases (Médiène et al. 2011) and reverse soil fertility decline to improve crop nutrient supply, especially nitrogen (N) (Lal and Stewart 2014). An approach that may contribute to achieve this goal is conservation agriculture (CA) including legumes. Conservation agriculture is based on three agronomic principles; minimum mechanical soil disturbance, permanent organic soil cover and the use of diverse crop rotations and/or associations (Hobbs et al. 2008). Gains in crop N supply under CA are intended to occur firstly, as a result of a decrease in N losses from leaching, runoff and erosion through soil protective measures and/or secondly, through an increased N input through N₂ fixation when legumes are included in crop rotations (Fageria et al. 2005; FAO 2011). On the other hand, surface application of mulch can result in increased gaseous N losses (Six et al. 2004). Along with gaseous N losses, immobilization of N in surface residues and decreased N mineralization rates can limit N availability and potentially decrease yields in no-till (Thierfelder and Wall 2011). In industrialized countries CA farmers have learned to cope with such crop N supply shortages by N fertilizer application. However, for smallholders in the tropics this option my not be feasible considering the little use of fertilizer N (Chianu et al. 2012). Moreover, tropical edaphic conditions can constrain legume symbiotic N₂ fixation (Giller 2001). For instance, soil acidity, calcium, magnesium, molybdenum or phosphorus deficiency can impair rhizobia survival (Hartwig 1998).

Although the potential challenges of CA are known, the role of legume N in CA established in tropical low input cropping systems on highly weathered soils remains unclear. Most studies on CA including legumes in this context have focused on maize - grain legume rotations and the effects of soil management, i.e., tillage vs. no till, on soil and water conservation and crop yields (Nyagumbo et al. 2016; Rusinamhodzi et al. 2011; Thierfelder et al. 2013). Investigations that addressed N dynamics in such a CA system and obtained positive effects on crop N uptake and yield (e.g., Maltas et al. (2009); Mhlanga et al. (2015)) could not conclusively relate these effects to the N input from the legume, because they did not differentiate between crop N derived from the soil or the legume. The use of ¹⁵N isotope techniques, which allow following the fate of residue N in the soil and quantifying its uptake by the subsequent crop (Hauck and Bremner 1976; Hood et al. 2008) combined with measurements of legume N₂ fixation, and of residual N in the soil could improve our understanding of benefits from legume N in these CA systems.

Experiments conducted in Madagascar to increase crop yield with little external inputs on highly weathered soils led to the introduction of the perennial forage legume Stylosanthes guianensis (stylo) into a CA crop rotation with upland rice (Husson et al. 2006). In this system, stylo is established as a relay crop with rice, growing simultaneously between rice rows. After rice harvest, stylo continues growing. Stylo shoot biomass is then cut and applied as mulch and the next rice crop is directly sown into the mulch. Yield improvements have been reported for rice and maize under CA with stylo after six years of conversion compared to yields under conventional practice in field experiments in the Midwest of Madagascar, but were mainly explained by lower weed (Striga asiatica) infestation under CA (Michellon et al. 2011). However, no study exists that follows symbiotic N₂ fixation by stylo and the transfer of stylo N to soil and the subsequent crop to show the assumed improvement in crop N supply after stylo fallow in this system.

Indeed, although stylo belongs to the tropical forge legume species most cited in the literature from sub-Saharan Africa (Agishi 1991; Thomas and Sumberg 1995), its N effect remains poorly quantified and few publications address the effect of stylo on rice N supply when used as a rotation crop in rainfed rice cropping systems. For example, stylo as a short term (four to six months) crop grown in Sub-Saharan Africa produced 1 to 17 Mg ha⁻¹

of shoot dry matter (DM) and took up 40 to 200 kg N ha^{-1} of which 63 to 79% was derived from the atmosphere, depending on rainfall and the soil fertility status (Akanvou et al. 2002; Becker and Johnson 1998; Ojiem et al. 2007; Saito et al. 2010). In the savannahs of West Africa, rice yields following stylo increased on average by 79% (Becker and Johnson 1998) and 41% (Saito et al. 2010) as compared to a weedy fallow, while Akanvou et al. (2002) reported no significant effect on subsequent rice yields. The reasons for such contrasting crop yield responses are not clear. According to Becker and Johnson (1998) rice grain yield was positively correlated with the N accumulation in stylo, which was greater where stylo biomass was incorporated into the soil than where stylo biomass was burnt prior to rice sowing. However, although these authors used a ¹⁵N isotope technique to determine the amount of N₂ fixed in stylo, they made no further use of ¹⁵N techniques to trace the fate of residue N in the soil and its uptake by the subsequent crop. Indeed, reported research results were mostly incomplete, making it difficult to relate stylo residue N to rice yield. For example, none of the studies presented rice N uptake, some even did not report the actual N in stylo biomass that was applied. Only Saito et al. (2010) measured the total and mineral soil N pool in the subsequent rice-cropping season, as an additional indicator for stylo N supply to rice, but found no significant effects of fallow and tillage management on these pools. Lastly, none of the studies quantified stylo root biomass, which is considered a substantial N source from legume residues (Peoples et al. 2001) and none of them studied stylo contribution to rice N supply under CA management (i.e., with no tillage and superficial legume residue restitution).

An overall picture on the supply of legume N to crops grown under CA, including the soil N dynamics as affected by the integration of legumes in CA, is lacking. To enhance our understanding on legume N supply to rice in low input tropical upland cropping systems under CA we established a field trial on a highly weathered Ferralsol in Madagascar. We used ¹⁵N isotope techniques to study how stylo grown as a relay crop in a CA based rice cropping system affects under different fertilizer regimes (1) the amount of N₂ fixed in stylo, (2) the rates of stylo mulch and root residue decomposition and N release and (3) the recovery of stylo mulch N in soil N pools and the succeeding rice crop.

Materials and methods

Study site description

The experimental site was located next to the village of Ivory (19°33'36.1" S, 46°24'34.7" E) at an altitude of 932 m in the region of Vakinankaratra, Madagascar. It was established on an arable field of approximately 0.5 ha, which had been hitherto farmer-managed and used for cropping. Subsistence agriculture is the main activity in this densely populated area of the Midwest Highlands. Farmers typically have about 1 ha of lowland where they grow irrigated rice and 0.5 to 2 ha of upland called `tanety` for rainfed crop production. Most farmers have also a few cattle (Bos indicus) used as draught animals. According to Koppen-Geiger classification (Peel et al. 2007), the climatic conditions correspond to a tropical savannah climate. On average the region receives 1300 mm of rain per year and the mean annual temperature is 23 °C. Annual rainfall has a wide year-to-year variation (decennial (2004-14) annual rainfall range 870 to 1800 mm) due to tropical cyclone and storm formations, which mainly hit Madagascar during the cyclone period from January to March (WMO 2015). Usually, the first rains fall erratically in mid-October and November, often followed by a short dry spell, before the main rainfall occurs from December to March (cropping season) and again erratic rains in April and May mark the end of the rainy season. During the field trial the total annual rainfall was 1800 mm (2010-11), 980 mm (2011–12) and 1450 mm (2012–13) (Fig. 1). The soil has been classified as haplic Ferralsol (Jones et al. 2013; WRB 2014) and has a sandy clay loam texture with 26% clay, 25% silt and 49% sand in the top soil layer (0 cm to 20 cm). Total N, phosphorus (P) and organic carbon concentration in the topsoil were 0.8, 0.4 and 10.8 g kg⁻¹ soil and the soil pH (H₂O) was 4.9 when the field experiment started.

Experimental design and treatments

The field trial was arranged as a completely randomized block experiment with four replicates. Treatment plot size was 6 m \times 10 m (hereafter referred to as main plots). In the crop rotation upland rice (NERICA 4, WAB 450-I-B-P-91-HB) was relay cropped with the forage legume *Stylosanthes guianensis cv.* Pucallpa (CIAT 184) (stylo) in cropping season one (Dec. 2010 to Mar. 2011), followed by one and a half year of stylo (Apr. 2011 to



Fig. 1 Cumulative rainfall (mm) during the first (2010–11), second (2011–12) and third (2012–13) rainy season at the field trial site next to the village of Ivory, Madagascar. The black square

indicates the sowing date of the rice crop in the first and third rainy season, while the black dot shows the date during the first rainy season when stylo was introduced into the standing rice crop

Oct. 2012), before in the third cropping season (Dec. 2012 to Mar. 2013) rice was again relay cropped with stylo. Treatments in this CA rotation (rice/stylo – stylo - rice/stylo) comprised the application of no fertilizer (NON), farmyard manure (FYM) or mineral fertilizer (MIN) to rice (Fig. 2). The respective acronyms were CA-NON, CA-FYM and CA-MIN. In addition to the CA rotation, a non-CA treatment termed MC-NON was established as an indicator of general/indigenous soil fertility. In the MC-NON treatment rice was grown without fertilizer as a mono crop (MC) in cropping season one and three, while in between the treatment plots remained as bare fallow (Fig. 2).

Crop management

At the start (November 2010) soil in all plots was tilled prior to sowing. As commonly practiced in this study area, tillage was conducted by hand using a long bladed version of the traditional spade `*angady*`, which allows turning the soil down to a depth of 20 cm. In December 2010, five to seven rice seeds were sown by hand in pockets with an inter-row spacing of 20 cm and an intrarow spacing of 30 cm. Rice seeds were protected from insect attacks by treating the seeds with insecticide (Insector T 45 DS ®, 35% imidacloprid and 10% thirame at 4 g kg⁻¹ seeds). Stylo seeds were sown 14 days after rice at a rate of 2 kg ha^{-1} in between the rice rows (spacing rice - stylo - rice: 15 cm) with an inter-row spacing of 40 cm between stylo pockets. This time lag of two weeks minimizes competition with upland rice while optimizing establishment and biomass accumulation of stylo (Shelton and Humphreys 1975). Seeding pockets for the rice and stylo seeds were prepared by using a narrow bladed version of the aforementioned angady as dibble stick. To break seed dormancy stylo seeds were submerged for 10 min in hot (80 °C) tap water (Nan et al. 1998). Neither soil liming, irrigation, nor rhizobia inoculation of stylo seeds or the soil were conducted, following





farmers practise. Rice received either no fertilizer, farmyard manure (5 Mg ha⁻¹) or mineral fertilizer as NPK (kg ha⁻¹ 33 N, 29 P, 40 K) and urea $(37 \text{ kg N ha}^{-1}, \text{ at panicle initiation})$. Weeding was conducted manually 21 days after sowing and at panicle initiation of rice. At rice harvest (March 2011), rice straw was distributed on the soil surface after threshing and stylo continued to grow. Weeding was conducted in all plots once after the onset of the major rains (December 2011) to promote stylo growth and canopy closure. Prior to the start of the third cropping season (October 2012), i.e., 23 months after establishment, stylo shoot biomass was cut using an angady at ground level. All cut stylo aboveground biomass (including litter) was retained in the plots. To have the same amount of stylo shoot biomass in each plot, stylo shoot biomass (litter excluded) was adjusted to 9 Mg DM ha⁻¹. Additional shoot biomass was taken from plots with the same cropping history, but not designated for stylo residue retention (Zemek 2016). After 14 days of drying in the sun, stylo shoot biomass (litter excluded) was chopped into 10 cm to 15 cm large pieces using knifes (hereinafter referred to as stylo mulch) and uniformly distributed on the plot surface. In December 2012, rice was directly sown through stylo mulch using an angady as dibble stick. Stylo regenerated from soil seed bank as relay crop with rice. All other crop management practices were as described for season one.

litter (j) biomass was sampled as indicated. At the end of the second dry season (23 months) all aboveground stylo biomass was cut (c) at ground level and applied as mulch and rice was sown through the mulch into non-tilled soil. Litter bags (lb) with stylo shoot and root residues were sampled at 42, 92 and 118 days after their amendment. Soil sampling (z) of the 0 to 10 cm soil layer was conducted at -5, +2, +43, +73, +93 and +117 days after the sowing (DAS) of rice. At +117 DAS soil was addditionally sampled from the 10 to 20 and 20 to 30 cm soil layer

Determination of stylo above- and belowground biomass production

Stylo shoot biomass was determined after four, 17 and 23 months of stylo growth (Fig. 2). To this end, stylo shoots from two times 1 m \times 1 m per plot were cut at ground level. Litter (fallen leaves and inflorescences) was collected from the same area and cleaned from adhering soil particles at the sampling after the dry season in October 2012. Shoot biomass and litter from each sampled square was weighed and DM content determined by drying a subsample at 60 °C for 48 h.

Stylo roots were sampled at the end of the first and second cropping season (March 2011, 2012), when also shoot biomass was sampled (Fig. 2). Roots were sampled at 10 cm depth increments down to a soil depth of 30 cm. using an auger (Eijkelkamp, Giesbeek, the Netherlands, ø 8 cm). Soil cores were taken at four adjacent positions describing a rectangular pattern; i.e., directly above the cut plant and at half the inter-row, inter-line and inter-line-diagonal distance to the next plant. Roots were separated from soil by wet sieving, as described below. In season two, when stylo grew alone, stylo roots were sampled as follows: In each plot, at two different locations, one stylo plant was randomly selected. A frame of 40 cm \times 40 cm was placed at equidistance (i.e., each side had a distance of 20 cm distance to the stem) around the stem of the selected stylo plant. The stylo plant shoot was cut and three soil monoliths of the size 40 cm \times 40 cm \times 10 cm were consecutively excavated to a depth of 30 cm using a spade. Soil monoliths from the same sampling depth per plot were pooled, before roots and organic debris were separated from soil by sequential wet sieving (ø 5-, 2- and 0.5- mm mesh size). Roots retained in the different sieves were pooled and total root DM determined after drying the roots at 60 °C for 48 h. The proportions of stylo and rice roots in the retrieved root samples (average ratio 1:3) were determined following the DNA based molecular approaches of Linder et al. (2000) and Mommer et al. (2008).

Quantification of N2 fixation by stylo

The proportion of N in stylo derived from the atmosphere (%Ndfa) was determined using the natural abundance method (Amarger et al. 1979). The method could be applied since the natural enrichment of ¹⁵N in our soil relative to atmospheric N2 was according to Cadisch et al. (2000) sufficiently high (10 to 12%), with little variation in depth to 1.4 m (Supplementary information, Fig. S1) and in horizontal direction across all four blocks. Non-N2 fixing reference plants comprised nonlegume (mainly dicotyledonous) weeds and rice. For each sampled stylo plant, two weeds or, in the case of relay cropped stylo, one rice plant and one weed were chosen. Plant sampling was conducted towards the end of each cropping season (March), i.e., flowering to early seed setting of stylo (Fig. 2). In each of the four replicate plots per treatment, five stylo plants together with the two respective reference plants were harvested along a W-shaped pattern. All plants were cut at ground level and dried at 60 °C for 48 h.

Assessment of stylo residue decomposition and N release

The litterbag study conducted in the third cropping season (November 2012 – March 2013) was designed so that three litterbags were placed into each plot, corresponding to three sampling dates, i.e., 42, 92 and 118 days after their amendment. Stylo mulch litterbags were placed in horizontal position between rice rows on the soil surface under a layer of stylo mulch on soil surface. Root litterbags were buried at random locations in the soil in an upright position, spanning from 0 cm to 30 cm soil depth. Treatments included all CA-treatments, resulting in a total number of 72 litterbags. Stylo

residues were derived at the end of the dry season from stylo plants, which had been continually grown in the field for 23 months (Fig. 2). Both residue types were dried at 60 °C for 48 h prior to use and subsamples were taken to determine their initial chemical composition (Table 1). In total 18 g of dried stylo mulch with a N concentration of 8 g N kg⁻¹ DM was placed inside each litterbag (20 cm \times 20 cm nylon bags, mesh size 1.5 mm). Bag dimensions and sample size corresponded to a stylo mulch application rate of 4.5 Mg DM ha⁻¹ and 37 kg N ha⁻¹, i.e., half the total amount of stylo mulch applied in plots. For stylo root residues a representative portion of 10 g root DM with a N concentration of 10 g N kg⁻¹ DM was placed into each litterbag (8 cm × 30 cm). Amounts of stylo root DM were based on field observations made during root excavation and corresponded to a stylo root application rate of 1 Mg DM ha^{-1} and 10 kg N ha^{-1} . Upon litterbag removal, roots that had grown into the bags were removed by handpicking. After opening the bags stylo mulch or root residue DM was briefly rinsed with tap water to remove any adhering soil particles. Residues were dried at 60 °C for 48 h and weighed to determine the percent mass loss. To correct the weight of the plant residues sampled for C and N analysis for contamination with soil, subsamples (500 mg) were incinerated at 550 °C for 4 h to determine the ash-free dry mass content.

Assessment of stylo mulch N recovery by rice

To quantify stylo mulch N recovery by rice and in soil N pools, ¹⁵N labelled stylo was applied in micro plots $(1.8 \text{ m} \times 1.6 \text{ m})$ confined with rectangular metal sheet frames that were installed to a depth of 30 cm within the respective main plots. The ¹⁵N enriched stylo mulch was produced during the fallow season (2011-12). Therefore, 60 atom% ¹⁵N enriched (NH₄)₂SO₄ was dissolved in tap water and applied at a rate of 9 kg N ha^{-1} to the soil surface between the stylo plants with a watering can. Simultaneously, sucrose (C:N ratio of 10:1) was applied, in order to promote homogenous soil N labelling through microbial turnover of the applied label (Hood et al. 2000). Following Douxchamps et al. (2011) to minimize leaching by the heavy rains the applied dose was split. The first dose was given at the start of the second cropping season (December 2011) and the second dose was applied three months later (February 2012). The resulting ¹⁵N abundance of stylo N was 0.68 atom%. These stylo residues were then used

Residue type	DM	N conc.	C conc.	C:N	DM [¶]	N applied [¶]
	g litterbag ⁻¹	mg N g^{-1} DM	mg C g^{-1} DM	mass ratio	g DM m ⁻²	g N m ⁻²
Mulch [†]	18	8.3 (0.64)	451 (18.2)	55	450	3.7
Root [§]	10	10.2 (0.89)	447 (18.5)	44	100	1.0

Table 1 Initial quantities and chemical composition of stylo residue dry matter (DM) placed in litterbags and corresponding residue application rates per m^{-2}

Means $(n = 4) \pm$ standard deviation (SD)

[†] Stylo mulch residues chopped up with a knife in 10 to 15 cm pieces

[§] Representative mixture of stylo root DM of four root diameter sizes, i.e., 0.5 to 2.0 mm, 2.0 to 4.0 mm, 4.0 to 10.0 mm and >10.0 mm, cut into 3 to 5 cm long pieces using scissors

[¶]Areal density transformed rates of DM and N applied with stylo mulch and root residues in litterbags

to replace non-¹⁵N labelled stylo mulch in micro plots, which contained non-¹⁵N labelled stylo roots, litter and seeds. The stylo mulch was applied at a rate of 9 Mg DM ha⁻¹. It had a C:N mass ratio of 57 and 7.6 g N kg⁻¹ DM, resulting in 6.9 g N m⁻², of which 78 mg N kg⁻¹ DM was mineral N.

Subsequently, rice amended with ¹⁵N labelled stylo mulch was grown. At rice maturity, all rice plants were cut at ground level for yield determination. Rice grains (with hull, i.e., paddy rice) were manually separated from straw with a pedal powered rice thresher. Subsamples of rice grains and straw were dried at 60 °C for 48 h for DM determination. To account for border effects within micro plots, rice plants from the centre were separated from those of the two border rows and discarded after dry weight determination (Dourado-Neto et al. 2010). To calculate rice ¹⁵N recoveries from stylo mulch (see below) per micro plot we assumed the same ¹⁵N enrichment in rice DM from the border rows and the centre.

Assessment of stylo mulch N recovery in soil N pools

Soil sampling of the 0 cm to 10 cm soil layer was conducted six times during the rice cropping season (Fig. 2). Sampling dates were chosen according to critical rice development stages (Yoshida 1981), i.e., at 5 days before sowing (-5) and +2 (germination), +43 (tillering), +73 (panicle initiation), +93 (late flowering) and +117 (maturity) days after sowing of rice. At rice maturity (+117) soil samples were additionally taken from the 10 cm to 20 cm and 20 cm to 30 cm soil layer. Five soil samples were taken at random locations within each micro plot with a soil auger (\emptyset 1.8 cm, Pürkhauer, Eijkelkamp, Netherlands). The five soil cores from each layer were bulked, homogenised by mixing and a subsample was taken. Subsamples from the topsoil layer (0 cm to 10 cm) designated for soil mineral and microbial biomass N measurements were immediately placed in cooling boxes for transport and subsequently stored on station at 4 °C till further analyses (described below). All other soil samples were dried at ambient temperature and homogenised by sieving (2 mm).

Soil microbial biomass N was measured within one week after sampling using the chloroform fumigationextraction method (Brookes et al. 1985; Vance et al. 1987). All extracts were collected in 60 ml polypropylene bottles, frozen at -20 °C and shipped to Switzerland. In Switzerland, the samples were unfrozen and the total soluble N in fumigated and non-fumigated extracts was determined using a TOC/TN analyser (Dimatec DIMA-TOC 100, Germany). Microbial N was then estimated from the difference between N extracted from the K₂SO₄ extract of fumigated and non-fumigated soils, expressed as mg N kg⁻¹ oven dry soil. No correction factor was used to account for incomplete microbial cell lysis (Joergensen and Mueller 1996). To determine soil mineral N, K₂SO₄ extracts from non-fumigated soil were additionally analysed colorimetrically using a continuous flow injection analyser (SKALAR® San++ System, Netherlands).

¹⁵N analyses of microbial and mineral N were conducted in soil sample extracts from +2, +43, +73 and +93 days after sowing rice sowing. The ¹⁵N abundance in microbial N was determined by oxidizing fumigated and non-fumigated K₂SO₄ soil extracts (Cabrera and Kissel 1989; Koroleff 1983), followed by the application of the diffusion method of Goerges and Dittert (1998) as modified by Mayer et al. (2003). To measure the ¹⁵N abundance in mineral N, extracts from nonfumigated soil were subjected to the same diffusion method. In brief, during the oxidation step total soluble N in soil extracts is oxidized to NO_3^- , while the principle of the diffusion method is that NH_4^+ and NO_3^- are converted into volatile NH_3 , which is then trapped by an acidified filter and analysed for ¹⁵N on a mass spectrometer. After drying (72 h), filters were encapsulated in tincapsules prior to analysis of ¹⁵N isotopic composition.

Dried soil and plant sample processing and analyses

From each dried sample a representative subsample was shipped to Switzerland. There plant subsamples were milled with a micro hammer mill (Culatti®, Switzerland) and an ultra-centrifugal mill (Retsch® ZM 200, Germany) to a final size of 0.2 mm. Dried soil samples were milled to a fine powder with a ball mill (Retsch MM 200, Germany. Analyses for total N and C content were conducted on a NCS analyser (Flash EA 1112 series, Thermo Electron Corporation, USA). The ¹⁵N isotopic composition was determined on a stable isotope mass spectrometer (Europa Scientific, Crewe, UK) at the Stable Isotope Facilities, Department of Soil Science, University of Saskatchewan, Canada.

Calculations

The abundance of 15 N in a compartment expressed in atom% was calculated as

¹⁵N compartment_i =
$$\frac{{}^{15}N}{({}^{15}N + {}^{14}N)} x \ 100$$
 (1)

where i refers to the specific measured compartment.

The natural ¹⁵N abundances are expressed as a per mil (‰) deviation from that of atmospheric N₂, i.e., δ^{15} N (Unkovich et al. 2008):

$$\delta^{15}N(\%) = \frac{\operatorname{atom}\%^{15}N \operatorname{compartment}_{i} - \operatorname{atom}\%^{15}N \operatorname{standard}}{\operatorname{atom}\%^{15}N \operatorname{standard}} x \,1000\tag{2}$$

where N standard represents the proportion of 15 N in the atmosphere (0.3663 atom% 15 N).

The %Ndfa for each stylo plant was derived using the average δ ¹⁵N of two paired reference plants. The %Ndfa was calculated according to Shearer and Kohl (1986):

$$\% Ndfa = \frac{\delta^{15} \text{N reference plant} - \delta^{15} \text{N stylo}}{\delta^{15} \text{N reference plant} - B \text{ value}} \times 100 (3)$$

where reference plant refers to the selected non-N₂ fixing reference plant grown on the same soil and the B value is a correction factor for the within-plant fractionation of ¹⁴N and ¹⁵N between shoots and nodulated roots (Unkovich and Pate 2000). To determine the B-value a pot experiment was conducted (November 2012 to April 2013) in a glasshouse at the research facilities of the radioisotope laboratory (LRI), University of Antananarivo, Madagascar (described in further detail in the supplementary information and Fig. S2). It resulted in a B value of δ^{15} N -1.19 (SD ± 0.15) ‰, which agreed well with B values published for *Stylosanthes* spp. by

Yoneyama et al. (1986) (-0.74, Townsville stylo) and by Nguluu et al. (2001) (-1.60 to -1.86, stylo hamata). When mature rice plants were used as reference plants a weighted mean for the δ^{15} N was calculated from straw and grain according to Danso et al. (1993). For each plot the average %Ndfa of the five stylo plants was used to calculate the amount of N₂ fixed in stylo shoots from the atmosphere and for statistical analysis. The amount of N₂ fixed in stylo shoots from the atmosphere in kg N ha⁻¹ was calculated by multiplying the %Ndfa by the total amount of N (kg N ha⁻¹) contained in the stylo shoot biomass sampled at the end of each cropping season (March). This sampling time corresponded always with the flowering to early seed setting phase of the perennial stylo.

The percentage (PR) of stylo residue DM and its N content remaining in each litterbag were used to calculate the total DM and N release from each litterbag (Cobo et al. 2002);

$$PR(\%) = \frac{X_t}{X_0} x \, 100 \tag{4}$$

$$Total DM/N_{release} = \frac{X_0 x PR}{100}$$
(5)

where X_t is the dry weight (in g DM) or N content (in g N) at each sampling time and X_0 the initial dry weight or N content. The N content was calculated by multiplying the remaining DM with its N concentration.

Decomposition rate constants (k) were calculated using regression of \log_e percentage mass remaining on time (t) in days (Anderson and Hetherington 1999);

$$log_e\left(\frac{X_t}{X_0}\right) = -kt \tag{6}$$

Using ¹⁵N labelling the proportion of N derived from stylo mulch (%Ndfm) in a compartment was calculated after Hauck and Bremner (1976):

$$\% Ndfm = \frac{AE_{+N}}{AE_M} x \ 100 \tag{7}$$

where AE_{+N} is the atom% ¹⁵N excess of N in a compartment that received the ¹⁵N labelled stylo mulch and AE_{M} is the atom% ¹⁵N excess of N in the stylo mulch. The AE_{+N} was calculated according to Hart and Myrold (1996):

$$AE_{+N} = {}^{15}N \ compartment_i - {}^{15}N \ compartment_{control}$$
(8)

where compartment_{control} denotes the natural ¹⁵N abundance (atom%) in a soil N pool obtained under identical experimental conditions but without the application of ¹⁵N labelled stylo mulch. The AE_{+N} for rice shoots was calculated as weighted AE_{+N} considering grain and straw.

The AE_{+N} of soil microbial biomass N (SMBN) was calculated using a mass balance (Douxchamps et al. 2010; Mayer et al. 2003);

$$AE_{+N} SMBN = \frac{\left(TN_F x AE_{+N(F)}\right) - \left(TN_{NF} x AE_{+N(NF)}\right)}{(TN_F - TN_{NF})} x 100$$
(9)

where TN_F and TN_{NF} stand for total N concentrations (mg N kg⁻¹ soil) measured in extracts from fumigated and non-fumigated soil samples, respectively.

The amount of N in a compartment derived from stylo mulch (Ndfm) was calculated as;

$$Ndfm = \frac{\% Ndfm_{compartment_i}}{100} x \ TN_i \tag{10}$$

where TN is total N in a soil or plant compartment expressed in mg N kg⁻¹ soil or g N m⁻², respectively. TN was calculated as the product of the concentration of N in the compartment and its weight in g m⁻² (for plants) or mg kg soil⁻¹ (for soil). For soil, the weight of each layer was calculated by multiplying its volume for a 1 m² surface by the bulk density. The amount of N derived from the soil in a compartment was calculated by the difference between TN and the Ndfm. The N recovery in % of added stylo mulch (%MNRec) in a compartment was calculated according to Hauck and Bremner (1976);

$$\% MNRec = \frac{Ndfm_{compartment_i}}{TN_M} x \ 100 \tag{11}$$

where TN_M is the amount in g N m⁻² of applied stylo mulch N.

Statistics

All statistical analyses were carried out using R version 3.0.0 (R-Core-Team 2013). CA treatments (CA-NON, CA-FYM, CA-MIN) were compared using one-way analysis of variance (ANOVA), including blocks as random factor. Post-hoc analyses were done by Fisher's least significant difference test at the $\alpha = 0.05$ level of significance corrected for the number of comparisons

made (Bonferroni correction). Standard errors of the difference in means were calculated from the respective ANOVA. To assess the factor time for labile soil N pools and stylo residue decomposition parameters, sampling dates were compared in a one-way repeated-measures ANOVA using the multilevel approach (i.e., the lme() function and setting the method to ML = maximum likelihood, specifying block and sampling date as random effect). To determine if the mean of the bare fallow MC-NON treatment was significantly different from the mean of the non-amended CA treatment (CA-NON) we performed Welch's two sample t-test. Relationships between variables were determined using Pearson's correlation coefficient. Prior to analysis data was checked for normal distribution using the Shapiro-Wilk test and for homoscedasticity, using and the Levene's test. When assumptions were violated, data was analysed using Wilcox (2012) robust methods (WRS package, version 0.3-1) with trimmed means (0.2).

Results

Stylo above- and belowground biomass production

During establishment as relay crop with rice, stylo growth was slow with little biomass production, in contrast to the fallow season when shoot biomass increased by a factor of up to 20 (Table 2). After four months as relay crop, stylo in the CA-MIN treatment produced 1 Mg DM ha⁻¹, which was twice the amount of shoot biomass produced in the CA-NON treatment. Towards the end of the second rainy season (March 2012) stylo shoot biomass yields were similar for all CA treatments with an average of 10 Mg DM ha⁻¹ (Table 2). Total aboveground biomass at the end of the second dry season (October 2012), i.e., when stylo was cut and applied as mulch, was on average 11.4 Mg DM ha⁻¹, of which approximately 25% was fallen stylo litter (Table 2).

Stylo root biomass was much less than aboveground biomass and was mainly concentrated in the top 0 cm to 10 cm. At each sampling (March 2011, 2012), at least 80% of root biomass was recovered in the top 0 cm to 10 cm soil layer (Supplementary information, Fig. S3). Total stylo root biomass in the upper 30 cm of soil was on average 0.5 Mg DM ha⁻¹ after four months of stylo growth, which was significantly (p < 0.01) lower than

the amount of 1.3 Mg DM ha^{-1} retrieved at the second sampling in season two (Table 2).

Stylo above- and belowground dry matter N concentrations

Plant components of stylo differed in their N concentration, which in turn varied over time (Table 2). Stylo shoot N concentration ranged from 8 to 27 g N kg⁻¹ DM, and decreased over time. The N concentration in stylo litter sampled after 23 months was on average 18 g N kg⁻¹ DM, which was greater than the N concentration in standing stylo shoot biomass at this same sampling date (Table 2). Average N concentration in roots was 10 g N kg⁻¹ DM in season one and 13 g N kg⁻¹ DM in season two (Table 2).

¹⁵N signatures of stylo and reference plants

Irrespective of the cropping season the ¹⁵N natural abundance of the reference plants was significantly (p < 0.05) higher than that of the respective stylo plant (Supplementary information, Table S1). The average δ^{15} N of the weedy reference plants was 9.2%, which was lower than the ¹⁵N signature of total soil N (10 to 12%), but higher than the ¹⁵N signature of the rice plants (δ^{15} N 7.5%). Stylo grown for four and 17 months had an average δ^{15} N of 2 and 4‰ (Table 2). The δ^{15} N of stylo grown as relay crop was on average significantly (p < 0.001) lower after four than after 17 months of growth (Table 2). In the second season, there was a residual effect of the fertilizer (applied to rice in season one) on the $\delta^{15}N$ of style, with the $\delta^{15}N$ of manureapplied style (CA-FYM) being significantly (p < 0.05) higher by 1 to 1.5% than the δ^{15} N of stylo grown in the CA-MIN and CA-NON treatment (Table 2).

Stylo N uptake, %Ndfa and N2 fixation

Stylo shoots accumulated up to 225 kg N ha⁻¹ (Fig. 3). The %Ndfa in stylo shoots ranged from 48 to 74%, with an average of 53% towards the end of the second rainy season (Table 2). Total amounts of N₂ fixed in stylo shoot biomass after 17 months ranged from 96 to 122 kg N ha⁻¹, which was more than ten times the amount of N₂ fixed after four months of stylo growth (Fig. 3). The type of fertilizer applied to rice in the first cropping season had a significant (p < 0.05) effect on the amount of N₂ fixed in relay cropped stylo grown for four and

Table 2I23months	bry matter of continu	(DM), h led grow	V concer th at the	ntration, field tri	¹⁵ N abun al site at I	dance, perc vory	ent N deriv	/ed from	the atn	osphere	(%Ndfa)	of living	and residu	al stylo ple	ant compoi	nents sar	npled after	4, 17 and
Season	End of cr	opping se	cason 1 (March 21	(110		End of cro	pping sea	son 2 (N	Aarch 20	12)		End of di (October	y season 2 2012)			Pre-cropf season 3 (Novemb	ing er 2012)
Months	4						17						23					
Treatment [§]	Shoot DM Mg ha ⁻¹	$\overset{\mathfrak{g}}{\overset{\mathfrak{g}}{\operatorname{kg}}_{-}} \overset{\mathrm{N}}{\operatorname{N}}$	δ ¹⁵ N [†] %0	Ndfa ^{##} %	Root [#] DM Mg ha ⁻¹	${ m N}$ g N kg^{-1}	Shoot DM Mg ha ⁻¹	kgN kgN	δ ¹⁵ N [†] %0	Ndfa## %	Root [#] DM Mg ha ⁻¹	$\overset{\mathfrak{g}_{\mathbb{N}_{-1}}}{\overset{N}{_{\mathbb{N}_{-1}}}}N$	Shoot DM Mg ha ⁻¹	${ m g~N~g^{-1}}$	Litter ^{††} DM Mg ha ⁻¹	$k_{\mathfrak{G}_{-1}}^{\mathfrak{G}_{N}}N$	Mulch [¶] DM Mg ha ^{−1}	N"¶ g N kg ^{−1}
CA-NON	0.5	26.2	2.0	65	0.5	9.8	9.8	20.0	3.4	57	1.3	11.8	8.9	7.8	3.0	18.4	6	7.4
CA-FYM	0.6	26.7	1.2	74	0.4	9.7	9.8	20.7	4.9	48	1.2	12.2	8.1	7.9	3.3	18.3	9	6.8
CA-MIN	1.0	27.6	2.4	59	0.6	10.2	10.7	21.1	3.9	54	1.3	14.0	8.3	7.7	2.7	18.5	6	6.8
Mean	0.7	26.9	1.9	66	0.5	9.9	10.1	20.6	4.1	53	1.3	12.7	8.4	7.8	3.0	18.4	6	7.0
SED	0.14	1.76	0.59	6.7	0.14	0.51	0.61	0.78	0.43	4.5	0.27	1.18	1.13	0.45	0.58	0.30	na	0.41
$\mathrm{LSD}_{0.05}^{\$\$}$	0.46	5.77	1.94	22.0	0.46	1.66	2.00	2.60	1.42	14.6	0.88	3.87	3.70	1.47	1.91	1.00	na	1.35
Source of v	ariation																	
Block	ns	su	su	su	us	*	us	*	*	*	su	su	ns	ns	us	ns	na	ns
Treatment	*	su	su	su	us	su	su	su	*	su	ns	su	ns	su	us	su	na	su
Means (n =	$(4) \pm \text{stanc}$	lard devi	ation (S)	D); Not (applicable	(na); signif	icance leve	l *0.05, *	*0.01, *	***0.001	; standard	error of c	lifference (SED); degr	ees of free	dom: Blo	ck = 3, Tre	atment = 2
CA = cot	servation a	agricultu	re; no fe	stilizer (NON), fai	myard man	ure (FYM)	, NPK +	urea (N	(NII								
§§Fisher`s	least signi	ficant di	fference	test at th	$\alpha = 0.0$	5 level of si	ignificance	corrected	l for the	number	· of compa	risons me	ide (Bonfer	roni)				
†δ ¹⁵ N exp	ressed as ¿	t per mil	(%o) de	viation fi	rom that o	f atmosphe	ric N2. At t	he end of	f the sec	sond dry	season (2)	3 months)) no measu	rement of s	tylo δ^{15} N	was con	ducted	
#Roots sar	npled from	n 0 cm to	30 soil	depth														
## Percent	of total N	derived	from the	: fixation	1 of atmos	pheric N2, 6	determined	using the	¹⁵ N n	atural ab	undance aj	pproach						
††Litter co	mprised fa	ullen leav	es and i	nfloresc	ences													
Stylo sho	ot residues	chopped	I up with 2 to the 3	a knife same cro	in 10 to 1:	5 cm pieces. tory, but no	. Stylo shoc t designate	ot biomas d for style	s (litter o mulch	excluded	 was adju tion 	sted to 9	Mg DM ha	⁻¹ by remo	ving or add	ding stylc	o shoot bior	nass taken
The N c	mcentratic	n was de	stermine	d from s	stylo mulc	h sampled s	shortly befo	ore rice so	owing	4								

17 months, which resulted in the following order of treatments: CA-FYM = CA-NON < CA-MIN (Fig. 3). Stylo roots recovered from the upper 30 cm of soil contained an additional 16 kg N ha⁻¹ after 17 months of stylo growth. Assuming the same %Ndfa in stylo shoots and roots of approximately 50%, this corresponds to 8 kg N ha⁻¹ of fixed N contained in stylo roots.

Residue decomposition and N release

Stylo mulch and root residue decomposition patterns differed, with the highest losses of stylo mulch occurring during the early phase and towards the end of the cropping season, while roots decomposed fastest during the early cropping season (Fig. 4). At the end of the cropping season on average 61% of stylo mulch and 70% of roots were decomposed. Decomposition constants (k) calculated from mass losses were on average higher for stylo roots than for mulch and half-life times were on average 94 (SD ± 40) days for stylo mulch and 72 (SD ± 31) days for roots (Supplementary information, Table S2).

Although residue N release patterns were similar to DM decomposition dynamics, the proportion of N released was higher for stylo roots than mulch throughout the cropping season (Fig. 4). On average 47% of N initially contained in stylo mulch and 72% of N contained in root residues had been released at the end of the experiment. This corresponded to an average daily N release of 15 (SD \pm 4) mg N m⁻² day⁻¹ for stylo mulch and 6 (SD \pm 1) mg N m⁻² day⁻¹ for root residues (Supplementary information, Fig. S5).

Stylo mulch residue N concentration increased with time, with a simultaneous decrease in the C:N ratio, while root residues showed no significant difference in comparison to their initial composition (Fig. 4). At the end of the cropping season the N concentration in mulch residue DM was 33 (SD \pm 8) % higher, while the C:N ratio had decreased from 55 to 36.

Effect of stylo mulch application on soil N pools

Overall soil microbial biomass N ranged from 6 to 20 mg N kg⁻¹ soil. For each treatment, microbial N varied little until rice tillering, but tended to decrease from panicle initiation until rice harvest when microbial N was lowest (Supplementary information, Fig. S6). Irrespective of sampling date, the MC-NON treatment had the lowest microbial N, which was on average 8 (SD \pm 1) mg N kg⁻¹ soil. Across sampling dates the



Fig. 3 Stylo shoot N uptake derived from the atmosphere (Ndfa) and the soil (Ndfs) 4 and 17 months after its establishment as relay crop with rice, determined using the ¹⁵N natural abundance approach. CA = conservation agriculture; no fertilizer (NON), farmyard manure (FYM) or NPK + urea (MIN). Error bars show ± 1 SE (n = 4). Different capital letters on top of stacked bars indicate

significant (p < 0.05) difference between treatments for total N uptake. Different small letters within stacked bars indicate significant (p < 0.05) difference between treatments for Ndfa. For Ndfs there were no significant differences between fertilizer treatments after 4 and 17 months, respectively

Fig. 4 Dry matter (DM) decomposition (a), N release (b), N concentration (c) and C:N mass ratio (d) of stylo mulch and root residues retrieved from litterbags after 0, 42, 92 and 118 days after amendment; Pooled data of all CA treatments are shown because there was no significant effect of fertilizer (applied to rice) treatment. Error bars show ± 1 SE (n = 12). Asterisks indicate significant (p < 0.05) difference between mulch and root residues (a., b.). Different capital letters indicate significant (p < 0.05)difference between sampling dates for mulch or roots residues (c, d)



microbial N was 50% higher in the CA-NON than in the MC-NON treatment.

The proportion of microbial N derived from ¹⁵N labelled stylo mulch (%Ndfm) in the CA-NON

treatment did not significantly vary during the study and was on average 12 (SD \pm 4) % of microbial N (Fig. 5). Corresponding amounts of microbial N derived from stylo mulch averaged 2 (SD \pm 0.6) mg N kg⁻¹ soil.



Fig. 5 Soil microbial biomass N (**a**) and mineral (NH_4^+, NO_3^-) N (**b**) in the 0 cm to10 cm soil layer at -5, +2, +43, +73, +93 and +117 days after rice sowing (DAS). Amounts of N derived from soil (Ndfs) and from stylo mulch (Ndfm) were determined at +2, +43, +73 and +93 DAS using ¹⁵N labelled stylo mulch. Error bars show ± 1 SE (n = 4). Asterisks indicate significant (* p < 0.05, **

On average 3 (SD \pm 1) % of ¹⁵N labelled stylo mulch were recovered in microbial N.

The soil mineral N pool was overall highest at the start of the cropping season, while it was lowest at rice tillering and at harvest. Mineral N ranged from 4 to 25 mg N kg⁻¹ soil, except for the CA-MIN treatment where fertilizer addition shortly before sampling resulted in mineral N values of up to 60 mg N kg⁻¹ soil, as expected (Supplementary information, Fig. S6). Throughout the cropping season mineral N was two to five times higher in the CA-NON than in the MC-NON treatment. The %Ndfm in mineral N of the CA-NON varied little during the cropping season and was on average 9 (SD ± 2) %, which corresponded to 2 (SD ±

p < 0.01) difference of microbial biomass and mineral N between the MC-NON (1) and the CA-NON (2) treatment per sampling date. Different letters indicate significant (p < 0.05) difference of microbial biomass or mineral N between sampling dates for the CA-NON (small letters) and MC-NON (capital letters) treatment

1) mg N kg⁻¹ soil (Fig. 5). The N recovery in mineral N fluctuated between 2 and 3% of applied stylo mulch N.

In bulk soil, the N recovery from ¹⁵N labelled stylo mulch was 39%. It was highest in the topsoil (0 cm to 10 cm) and decreased with soil depth (Table 4). At rice harvest total N recoveries from stylo mulch in the soil (0 cm to 30 cm) were similar in all CA treatments with an overall average of 64 (SD \pm 13) %.

Rice dry matter yield and N uptake

Grain yield was significantly affected by fertilizer application (Table 3). As expected it was highest (458 g DM m^{-2}) for the CA-MIN and lowest (158 DM m^{-2}) for the

Table 3 Rice grain and straw dry matter (DM) yield, N concentration and uptake harvested from micro plots in cropping season 3 (2012–13)

		Yield		N concentrati	on	N uptake	
		g DM m ⁻²		g N kg ⁻¹ DM	[$g N m^{-2}$	
Treatment [†]		Grain ^{††}	Straw	Grain	Straw	Grain	Straw
MC-NON [¶]		158 (77)	124 (48)	12 (0.9)	6 (1.2)	2 (0.8)	1 (0.8)
t - test (p) ¶		ns	ns	ns	*	ns	ns
CA-NON		271	175	11	4	3	1
CA-FYM		253	159	11	4	3	1
CA-MIN		458	281	14	6	6	2
Mean		327	205	12	5	4	1
SED		36	18	1	1	0.4	0.1
LSD _{0.05} ^{§§}		117	60	2	2	1.7	0.3
Source of variation	df						
Block	3	*	ns	ns	ns	ns	ns
Treatment	2	**	**	**	*	***	***

Means $(n = 4) \pm$ standard deviation (SD); Not applicable *(na)*; significance level *0.05, **0.01, ***0.001; standard error of difference (SED).

[†] MC = mono cropping, CA = conservation agriculture; no fertilizer (NON), farmyard manure (FYM) or NPK + urea (MIN)

^{§§} Fisher's least significant difference test at the $\alpha = 0.05$ level of significance corrected for the number of comparisons made (Bonferroni) ^{††} Paddy (grain and husk) yield data were adjusted to the standard moisture content of 0.14 g H₂O g⁻¹

 $\[\]$ Results for the MC-NON were not included in the ANOVA. Shown are the mean (\pm SD) and the t-test probabilities of significant difference to the mean of the CA-0 treatment

MC-NON treatment. The CA-NON treatment produced on average 60% more grain DM than the MC-NON treatment, although this effect was not statistically significant. Except for the CA-MIN treatment, N concentration showed minor variation among treatments and was on average 12 g N kg⁻¹ DM for rice grains and 5 g N kg⁻¹ DM for straw (Table 3).

Similar to DM yield, mineral fertilizer led to the highest N uptake (8 g N m^{-2}) in rice shoots, with all

other treatments ranging between 2.6 and 3.7 g N m⁻² (Table 3). Although not statistically significant, the amount of N derived from the soil was 30% higher in the CA-NON than in the MC-NON treatment (Fig. 6). The %Ndfm did not significantly differ between CA treatments and was on average 10 (SD ± 3) %, or 0.3 to 0.6 g N m⁻² (Fig. 6). On average 7% of stylo mulch N was recovered in rice shoots, with overall greater N recovery in grains than in straw (Table 4).

Fig. 6 Rice shoot (grain and straw) N uptake derived from stylo mulch (Ndfin) and from the soil and applied fertiliser (Ndfs + f) determined using ¹⁵N labelled stylo shoot residues. MC = mono cropping, CA = conservation agriculture; no fertilizer (NON), farmyard manure (FYM) or NPK + urea (MIN). Error bars show ± 1 SE (*n* = 4)



		¹⁵ N recovery						
		(% of stylo mulch a	added at day 0)					
		Surface mulch [§]	Soil			Rice		Total [¶]
Treatment [†]			0-10 cm	10–20 cm	20–30 cm	Grain [#]	Straw	
CA-NON		39	37	20	9	4	1	109
CA-FYM		44	40	18	8	4	1	114
CA-MIN		35	40	12	10	6	2	106
Mean		39	39	16	9	5	2	110
SED		8.4	5.0	3.7	1.5	0.9	0.2	6.6
LSD _{0.05} ^{§§}		27.6	16.5	12.1	4.9	3.0	0.6	21.6
Source of variation	df							
Block	3	ns	ns	ns	*	ns	ns	*
Treatment	2	ns	ns	ns	ns	ns	**	ns

Table 4 ¹⁵N recovery from ¹⁵N labelled stylo mulch at rice harvest

Means $(n = 4) \pm$ standard deviation (SD); Not applicable *(na)*; significance level *0.05, **0.01, ***0.001; standard error of difference (SED).

[†]CA = conservation agriculture; no fertilizer (NON), farmyard manure (FYM) or NPK + urea (MIN)

[§] Stylo mulch DM remaining at rice harvest in litterbag study; ¹⁵ N recovery determined mass balance approach

[#]Rice paddy (grain and husk)

[¶] Total recovery is the sum of recoveries in rice, soil (0 cm to 30 cm) and stylo mulch remaining on the soil surface at rice harvest

^{§§} Fisher's least significant difference test at the $\alpha = 0.05$ level of significance corrected for the number of comparisons made (Bonferroni)

Discussion

Stylo N accumulation

The total amount of stylo N accumulation mainly depended on shoot biomass production, since shoot N concentrations and the %Ndfa showed minor variation (Table 2). These results are in line with other studies, e.g., Cadisch et al. (1989) for eight tropical forage legumes including stylo, Ojiem et al. (2007) for stylo and Oberson et al. (2013) for clover. Stylo seems having benefited from the greater N availability in the mineral fertilizer amended rice treatment, as shown in a significantly higher stylo shoot biomass production and shoot N uptake during the first four months of fallow establishment, i.e., in the CA-MIN treatment as compared to the CA-NON and CA-FYM treatment (Table 2, Fig. 3). This suggests that fertilizer inputs to rice during stylo fallow establishment improve stylo growth and N uptake on highly weathered upland soils, as was probably the case for stylo in the CA-MIN treatment.

Somewhat surprisingly, stylo root biomass was low irrespective of treatment (Table 2), which suggests that

roots in contrast to shoots (shoot:root DM ratio of 8:1) play a minor part in the total N accumulated by stylo. The low root biomass may explain the importance of mycorrhiza fungi, which are naturally present in many field soils, in the nutrition and growth of stylo, in particular on marginal soils (Jehne 1984). After 17 months, stylo had accumulated on average 16 kg N ha⁻¹ in root DM, which was 12 to 15 times less than N taken up in shoot biomass. Our results are supported by the few studies, which assessed stylo root biomass. In a pot study on the effect of soil fertility and NPK application on stylo shoot and root DM production shoot to root ratios ranged from 3:1 (-NPK) and 9:1 (+NPK) (Tian and Kang 1998). Szott et al. (1994) quantified root biomass of stylo to a soil depth of 45 cm in managed fallow systems on a tropical Ultisol (Acrisol) in Peru with an annual rainfall of 2200 mm. At 17, 29, 41, and 53 months after fallow initiation root biomass irrespective of above ground biomass (8 to 18 Mg ha^{-1}) was less than 5 Mg ha^{-1} . In a study on the effects of P and K supply on growth and N₂ fixation of tropical foragelegumes cultivated in an Oxisol of the Eastern Plains of Colombia Cadisch et al. (1989) determined less than 5 kg N ha⁻¹ in stylo root biomass recovered from 0 cm to 20 cm soil depth.

The physical recovery of roots however, provides only a rough estimate of stylo root N and DM accumulation, since the method does not consider fine roots or seasonal rhizodeposition. In a review, Fustec et al. (2010) reported that legume rhizodeposition (decomposition and decay of nodules and root cells and exudation of soluble N compounds by plant roots) may comprise 4 to 71% of total plant N. In a meta-analysis of published data on N2 fixation in Australian crop and pasture legumes (Unkovich et al. 2010) calculated 'root factors' of 2.0 (Medicago sativa) and 1.7 (Trifolium subterraneum) to multiply shoot total N by to estimate shoot + root N. Using such a factor for the conversion of aboveground legume N to estimate total N accumulation would result in significantly higher N contribution of stylo roots. In our study, soil mineral and microbial N after stylo fallow were substantially greater in the CA-NON treatment than in rice mono cropped (MC-NON) soil. However, only a small proportion of the surplus in soil mineral and microbial N was derived from the ¹⁵N labelled stylo mulch (Fig. 5). This indicates that residual N of non-15N labelled stylo root N contributed to these pools over the following rice season, although we cannot entirely separate that effect from the N effect of non-15N labelled aboveground litter and seed fall during the dry season 2012 (see below).

Seasonal stylo residue N turnover and stylo mulch N recovery in soil N pools

Residue decomposition and N release patterns can mostly be explained by climatic conditions, although in the case of this study also the contact of the residues with the soil need to be considered (i.e., stylo mulch applied on the soil surface, root residues in soil). The high early residue N release (Fig. 4) is in line with other studies looking at residue decomposition in tropical agro ecosystems. For example, Thomas and Asakawa (1993) observed during the rainy season a release of 60% of stylo litter N after 14 days and 80% after 42 days. Such early N release and residue decomposition dynamics can be explained by the onset of the rains and resulting dry-wet cycles, which enhance physical breakdown of residues and promote microbial residue decomposition (Birch 1964).

Fungi may also have played a major role in stylo mulch turnover. At rice harvest about 70% from root

DM and N had been lost from the litterbags, while the DM loss (61%) from stylo mulch was higher than the proportion of N (47%) released (Fig. 4). Thus, N loss from stylo mulch was slower than loss of DM, resulting in increasing N concentrations in mulch residues with time (Fig. 4). The same phenomenon was also observed by Urguiaga et al. (1998), Salas et al. (2003), Hart et al. (1993), Frey et al. (2000) and Matos et al. (2011), who related the increase in N concentration to the decomposition of residues to fungal rather than bacterial decomposition. Fungi maintain a number of characteristics that present advantages over bacteria to decompose low quality residues placed on the soil surface (Rasche and Cadisch 2013). Most importantly, their extensive hyphae network allows fungi to utilize both the surface residue C and soil N and moisture (Beare et al. 1992; Holland and Coleman 1987; Yuste et al. 2011). Further, as hyphal growth proceeds, nutrients are immobilized in the hyphal cell walls where their potential for mineralization is low (Kassim et al. 1981). This indicates that the increase in N concentrations in stylo mulch residues at the later stages of decomposition could be due to immobilized N within stylo mulch residues related to colonisation by fungi, although a net N release occurred. Visual observations made at litterbag recovery that showed extensive fungal growth on shoot and root residues (Supplementary information, Fig. S7), support this assumption.

Overall, most of ¹⁵N labelled stylo mulch N (~ 64%) was recovered in the top 30 cm of bulk soil (Table 4). This high N recovery in the soil was consistent with other studies on organic residues in tropical agro-ecosystems (Dourado-Neto et al. 2010; Douxchamps et al. 2011) and indicates a build up of potentially mineralizable N in soil. However, in our study only 61% of the stylo mulch had been decomposed at rice harvest (Fig. 4), with the non-decomposed remainder, corresponding to 39% of ¹⁵N applied with stylo mulch, still on the soil (Table 4). We assume that this remainder contributes upon decomposition to the N input in soil from stylo mulch, though the extent may depend on the removal by soil macro fauna (Vanlauwe et al. 1998) and the occurrence of bushfires during the dry season (Kull 2016).

The fate of stylo residue N not recovered in the litterbags is not entirely clear. At rice harvest, most of ¹⁵N labelled stylo mulch N was recovered in the top 10 cm of soil (Table 4). However, little of this was present in the soil microbial and mineral N pools, in which together around 6% of stylo mulch ¹⁵N was

recovered. Likewise, only about 1% was recovered in the dissolved organic N pool (calculated as the difference between total soluble N (extracted with 0.5 M K_2SO_4) and soil mineral N (van Kessel et al. 2009) Supplementary information, Fig. S8). Therefore, we assume that most of the stylo mulch N transferred to the topsoil layer, but not recovered in specific soil N pools remained in the pool of non-decomposed residues, i.e., physically uncomplexed organic matter (light fraction; specific gravity 1.6 to 2.0 g cm⁻³) and particulate organic matter (> 53 to 2000 mm in diameter)) (Beare and Gregorich 2007), which may contain 13-40% of total soil N (Haynes 2005). Further research would be needed to characterize its form in order to evaluate its potential and temporal availability as N source for subsequent crops and the build up of soil N stocks. As discussed below, the total mulch N recovery suggests that little mulch N was lost from the fields.

Stylo mulch N uptake and recovery by rice

The immediate and long-term N benefit from legumes will depend largely on the proportion of legume derived N taken up by the succeeding crop and its recovery in the soil. Using ¹⁵N labelled stylo mulch showed that the %Ndfm in rice (grain and straw) was similarly low (~9%) in all treatments, irrespective of fertilizer application, with most N derived from the soil and fertilizer (Fig. 6). The corresponding 15 N recoveries in rice of 5 to 8% from stylo mulch (Table 4) were similar to N recoveries in maize (8.5%) from applied ¹⁵N-labelled legume (Leucaena leucocephala) residues in alley cropping systems in Nigeria (Vanlauwe et al. 1998) and in maize (7.3%) from applied ¹⁵N-labelled legume (Crotalaria juncea) residues in the Guinea savannah zone in northern Ghana (Fosu et al. 2003). In our study, the low stylo mulch N recovery in rice reflects the low mineralization of stylo mulch N as a result of stylo mulch placement on the soil and its C:N ratio of 55. However, despite the low N supply by stylo mulch to rice, stylo culture increased rice N uptake by an additional 30%, as shown in the difference in the amount of N derived from the soil in rice between the CA-NON and the MC-NON treatment (Fig. 6). Most likely, this reflects the greater (on average 75%) seasonal soil mineral N in the CA-NON than in the MC-NON treatment (Fig. 5) and in part explains the 70% increase in rice grain yield in the CA-NON treatment (Table 3). This may be because of stylo N contribution beyond that by ¹⁵N labelled stylo mulch, which is discussed below.

Total ¹⁵N recoveries slightly greater than 100% at rice harvest, in the remaining surface mulch, rice and soil down to 30 cm (Table 4), suggest that N losses from the system were likely to be minor. This could be linked to the stabilization of stylo mulch N derived particulate organic matter through biochemical stabilization and physical protection within soil aggregates (Six et al. 2002), leading to lower rates of mineralization and losses via leaching, which is particularly important under tropical rainfall conditions. The reason of total ¹⁵N recoveries greater than 100% of the total ¹⁵N added with stylo mulch may lay in the susceptibility of the ¹⁵N tracer technique to changes in soil bulk density although in this study outmost care was taken to accurately determine the soil bulk density in the different treatments.

Known and potential stylo N pools and flows

Stylo N related pool sizes changed over time. The following combination of measured and estimated stylo N pools and flows (Fig. 7) broadens our understanding of stylo N supply and identifies future research questions. At the end of the second cropping season (March 2012, 17 months of stylo growth) stylo had accumulated 195 to 225 kg N ha⁻¹ in shoot biomass (Fig. 3), suggesting a high potential N source for the following rice crop. However, only half of this N was found at the final harvest after the dry season (October 2012, 23 months of growth), with on average 65 and 55 kg N ha⁻¹ in stylo shoots and litter (Table 2). Clearly, the fate of N contained in stylo litter demands for further investigation, since also other authors (Becker and Johnson 1998; Muhr et al. 2002; Saito et al. 2010) did not pay attention to fallen leaves as potential crop N source. The lack of about 100 kg N ha⁻¹ from stylo biomass N after the dry season could be explained by plant internal translocation of accumulated N from vegetative to regenerative plant components (seeds) during the post-cropping season (Gardener et al. 1982; Vallis and Gardener 1984) and to drought tolerance and avoidance mechanisms that allow the plant to survive the long dry season (Fisher and Ludlow 1984; Gardener 1984; Little et al. 1984; Mott et al. 1981; Probert 1984). Indeed, stylo produces a high number of small seeds (230,000 to 1,250,000 seeds ha^{-1}), which depending on the species can amount to a total of 620 to 1763 kg ha⁻¹ (Hopkinson and Walker 1984). For the species Stylosanthes guianensis, 827 and



Fig. 7 The distribution and cycling of N accumulated by *Stylosanthes guianensis* (stylo) as a fallow crop in a three-year conservation agriculture based crop rotation with upland rice on a Ferralsol in the Midwest Highlands of Madagascar. Solid lines and solid rectangles show quantified N fluxes and distribution of total stylo biomass N into sub-pools. Dashed lines and dashed rectangles show estimated N fluxes and distributions. Circles represent

1000 kg ha⁻¹ have been reported by Gardener (1984). Assuming a seed N concentration of 58 g kg⁻¹ DM (determined in seeds of this study's B-value experiment), which is at the lower end of stylo seed N concentrations from 56 to 68 g kg^{-1} DM reported by Gardener et al. (1982), and a seed N production of 1000 kg ha^{-1} , suggests that 60% of the N not found in aboveground stylo biomass after the dry season could be related to seed formation. A large proportion of these seeds can rest in the soil (deLeeuw et al. 1992; McKeon and Mott 1984; Orr 2010). An evaluation of tropical pasture species as leys in the semi-arid tropics of northern Australia showed that seeds of stylo can establish a seed bank in the soil for over 4 years (Cameron 1996). Potential losses, for example of up to 40% of N removed by ants and termites were reported by McKeon and Mott (1984).

the main N pools; Quantities of fluxes, distributions, and pools are in kg N ha⁻¹; Figures in brackets show the amount of stylo N recovered in the respective N pool. Shape sizes do not correspond to N amounts. Gray scale in the background shows time. Rhizodeposition N estimate (range 4 to 71% of total plant N) are based on Fustec et al. (2010)

Whether the other missing 40% of N not found in aboveground stylo biomass after the dry season have been transferred to the root systems remains unclear, since roots were not sampled after the dry season. However, it seems realistic as shown in a pot study under drought conditions reported by Vallis and Gardener (1984), where the proportion of total plant N transferred into the roots can range from 13 to 34% of total plant N during flowering and seed setting. Thus, this translocation could have increased the potential amount of 17 kg N ha⁻¹ stored in root residues (only considering N concentration and amounts of DM determined in April 2012, Table 2) and its N release upon decomposition during the following rice season. As discussed above, the physical recovery of roots however neglects fine roots and seasonal rhizodeposition. Using allocation factors to

relate legume shoot N to rhizodeposition (Fustec et al. 2010; McNeill and Fillery 2008; Unkovich et al. 2010) would result in a significantly higher N contribution of stylo roots.

In sum, latter N fluxes and pools derived from N accumulated in stylo biomass after 17 months may explain why unexpected stylo mulch in contrast to other stylo N components (seeds, litter, roots) played a limited role for rice yield through its N supply. To properly understand short term yield increases through improved crop N supply other stylo N components (seeds, litter, roots) need to be studied in greater detail (using ¹⁵N labelling). Finally, since CA is considered a long term approach, there is also a need to study the effect of successive crop rotational cycles with stylo mulch application on crop N supply and yield through the build up of soil N stocks, as indicated by the high total N recoveries from stylo mulch remaining on the soil surface and in the soil at rice harvest.

Conclusions

This study has shown that increasing crop N supply and yield is possible through integration of legumes such as stylo in low input CA upland cropping systems on highly weathered tropical soils. Under the edaphic conditions of the Malagasy highlands stylo accumulated irrespective of fertilizer input substantial amounts of N in its aerial biomass, of which about half was derived from N₂ fixation. Yet, the contribution of the assumed major stylo N source, i.e., stylo mulch, to the following rice crop was small because its decomposition was incomplete and N released was mainly present in nonplant available form. The ¹⁵N labelled stylo mulch did not represent the total stylo N input, but only about 30% of stylo N present at 17 months of stylo growth. Such, stylo N released from litter and seeds fallen off during the dry season and from roots seems to be an additional important N source, which should be further investigated and be included in predictions on stylo N supply to crops. Higher mineral and microbial N pools after stylo fallow as compared to the rice mono crop treatment indicated that stylo litter, seeds and root residues increased the soil mineralization potential beyond the effect detected with the ¹⁵N labelled stylo mulch. High total N recoveries from stylo mulch remaining on the soil surface and in the soil can be important for the build up of soil organic N stocks, which are crucial to improve crop N supply and soil fertility in low input upland cropping systems on highly weathered tropical soils.

Acknowledgments The authors are grateful for funding from the Swiss National Science Foundation and the Swiss Agency for Development and Cooperation (Project no. IZ70Z0_131310/1). We acknowledge Adolphe Munyangabe (currently Department of Soil Sciences, FIBL, Switzerland) for lab assistance with sample preparation. Further, we warmly thank Agnès Nivotiana (FOFIFA, Antsirabe, Madagascar) for field trial maintenance and assistance with sample preparation. Appreciation is extended to Myles Stocki (University of Saskatchewan, Canada) for mass spectrometer measurements. Special thanks go to Gregor Meyer (Group of Plant Nutrition, ETH Zurich), for fieldwork assistance. The authors thank Prof. Lilia Rabeharisoa (LRI, Antananarivo, Madagascar) and Prof. Jacqueline Rakotondravelo (FOFIFA, Antananarivo, Madagascar) for their collaboration in project steering and provision of logistic support.

Authors' contributions OZ: Conception and design, fieldwork, laboratory analysis, data interpretation and analysis, manuscript writing; EF, AO, and ES: Conception and design, coordination, data interpretation and manuscript revision. All authors read and approved the final draft.

Compliance with ethical standards

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

References

- Agishi E (1991) A bibliography on forage legumes in Africa. Working doc no A1. Forage agronomy section. ILCA, Addis Ababa
- Akanvou R, Kropff MJ, Bastiaans L, Becker M (2002) Evaluating the use of two contrasting legume species as relay intercrop in upland rice cropping systems. Field Crop Res 74:23–36. https://doi.org/10.1016/S0378-4290(01)00198-8
- Amarger N, Mariotti A, Mariotti F, Durr JC, Bourguignon C, Lagacherie B (1979) Estimate of symbiotically fixed nitrogen in field-grown soybeans using variations in N-15 natural abundance. Plant Soil 52:269–280. https://doi.org/10.1007 /Bf02184565
- Anderson JM, Hetherington SL (1999) Temperature, nitrogen availability and mixture effects on the decomposition of heather [Calluna Vulgaris (L.) hull] and bracken [Pteridium Aquilinum (L.) Kuhn] litters. Funct Ecol 13:116–124. https://doi.org/10.1046/j.1365-2435.1999.00014.x
- Beare M, Gregorich E (2007) Physically Uncomplexed organic matter. Soil sampling and methods of analysis. CRC Press, Second Edition

- Beare MH, Parmelee RW, Hendrix PF, Cheng WX, Coleman DC, Crossley DA (1992) Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. Ecol Monogr 62:569–591. https://doi. org/10.2307/2937317
- Becker M, Johnson DE (1998) Legumes as dry season fallow in upland rice-based systems of West Africa. Biol Fertil Soils 27:358–367. https://doi.org/10.1007/S003740050444
- Birch HF (1964) Mineralisation of plant nitrogen following alternate wet and dry conditions. Plant Soil 20:43–49
- Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842. https://doi. org/10.1016/0038-0717(85)90144-0
- Cabrera ML, Kissel DE (1989) Review and simplification of calculations in15N tracer studies. Fertilizer Research 20:11– 15. https://doi.org/10.1007/bf01055396
- Cadisch G, Sylvester-Bradley R, Nösberger J (1989) 15N-based estimation of nitrogen fixation by eight tropical foragelegumes at two levels of P:K supply. Field Crop Res 22: 181–194. https://doi.org/10.1016/0378-4290(89)90091-9
- Cadisch G, Hairiah K, Giller KE (2000) Applicability of the natural N-15 abundance technique to measure N-2 fixation in Arachis Hypogaea grown on an Ultisol. Neth J Agr Sci 48: 31–45
- Cameron AG (1996) Evaluation of tropical pasture species as leys in the semi-arid tropics of northern Australia. Aust J Exp Agr 36:929–935. https://doi.org/10.1071/Ea9960929
- Chianu J, Chianu J, Mairura F (2012) Mineral fertilizers in the farming systems of sub-Saharan Africa. A review. Agron Sustain Dev 32:545–566. https://doi.org/10.1007/s13593-011-0050-0
- Cobo JG, Barrios E, Kass DCL, Thomas RJ (2002) Decomposition and nutrient release by green manures in a tropical hillside agroecosystem. Plant Soil 240:331–342. https://doi.org/10.1023/a:1015720324392
- Danso SKA, Hardarson G, Zapata F (1993) Misconceptions and practical problems in the use of N-15 soil enrichment techniques for estimating N2 fixation. Plant Soil 152:25–52. https://doi.org/10.1007/Bf00016331
- deLeeuw PN, Saleem MA, Nyamu AM (1992) Stylosanthes as a forage and fallow crop. In: PN deLeeuw, MA Saleem, AM Nyamu (eds) Proceedings of the Regional Workshop on the Use of Stylosanthes in West Africa held in Kaduna, Nigeria, 26–31 October 1992. International livestock Centre for Africa, Addis Ababa, Ethiopia
- Dourado-Neto D, Powlson D, Bakar RA, Bacchi OOS, Basanta MV, Cong P, Keerthisinghe G, Ismaili M, Rahman SM, Reichardt K, Safwat MSA, Sangakkara R, Timm LC, Wang JY, Zagal E, van Kessel C (2010) Multiseason recoveries of organic and inorganic Nitrogen-15 in tropical cropping systems. Soil Sci Soc Am J 74:139–152. https://doi.org/10.2136 /sssaj2009.0192
- Douxchamps S, Humbert FL, van der Hoek R, Mena M, Bernasconi SM, Schmidt A, Rao I, Frossard E, Oberson A (2010) Nitrogen balances in farmers fields under alternative uses of a cover crop legume: a case study from Nicaragua. Nutr Cycl Agroecosyst 88:447–462. https://doi.org/10.1007 /S10705-010-9368-2

- Douxchamps S, Frossard E, Bernasconi SM, van der Hoek R, Schmidt A, Rao IM, Oberson A (2011) Nitrogen recoveries from organic amendments in crop and soil assessed by isotope techniques under tropical field conditions. Plant Soil 341:179–192. https://doi.org/10.1007/S11104-010-0633-6
- Fageria NK, Baligar VC, Bailey BA (2005) Role of cover crops in improving soil and row crop productivity. Commun Soil Sci Plant Anal 36:2733–2757. https://doi.org/10.1080 /00103620500303939
- FAO (2011) Green manure/cover crops and crop rotation in conservation agriculture on small farms. Food & agriculture organization of the united nations (FAO)
- Fisher MJ, Ludlow MM (1984) 8 adaptation to water deficits in Stylosanthes. In: Stace HM, Edye LA (eds) The biology and agronomy of Stylosanthes. Academic Press
- Fosu M, Kühne RF, Vlek PG (2003) Recovery of cover-crop-N in the soil-plant system in the Guinea savannah zone of Ghana. Biol Fert Soils 39:117–122. https://doi.org/10.1007/s00374-003-0677-3
- Frey SD, Elliott ET, Paustian K, Peterson GA (2000) Fungal translocation as a mechanism for soil nitrogen inputs to surface residue decomposition in a no-tillage agroecosystem. Soil Biol Biochem 32:689–698. https://doi.org/10.1016 /S0038-0717(99)00205-9
- Fustec J, Lesuffleur F, Mahieu S, Cliquet JB (2010) Nitrogen rhizodeposition of legumes. A review. Agron Sustain Dev 30:57–66. https://doi.org/10.1051/agro/2009003
- Gardener CJ (1984) 17 the dynamics of Stylosanthes pastures. In: Stace HM, Edye LA (eds) The biology and agronomy of Stylosanthes. Academic Press
- Gardener CJ, Megarrity RG, Mcleod MN (1982) Seasonalchanges in the proportion and quality of plant-parts of 9 Stylosanthes lines. Aust J Exp Agr 22:391–401. https://doi. org/10.1071/Ea9820391c
- Giller K (2001) Nitrogen fixation in tropical cropping systems. Wageningen University, The Netherlands
- Goerges T, Dittert K (1998) Improved diffusion technique for 15N:14N analysis of ammonium and nitrate from aqueous samples by stable isotope spectrometry. Commun Soil Sci Plant Anal 29:361–368. https://doi.org/10.1080 /00103629809369950
- Hart SC, Myrold DD (1996) 15N tracer studies of soil nitrogen transformations. In: Boutton TW, Yamasaki SI (eds) Mass spectrometry of soils. Dekker Inc., New York
- Hart SC, Firestone MK, Paul EA, Smith JL (1993) Flow and fate of soil-nitrogen in an annual grassland and a young mixedconifer Forest. Soil Biol Biochem 25:431–442. https://doi. org/10.1016/0038-0717(93)90068-M
- Hartwig UA (1998) The regulation of symbiotic N2 fixation: a conceptual model of N feedback from the ecosystem to the gene expression level. Perspect Plant Ecol, Evol Syst 1:92–120
- Hauck RD, Bremner JM (1976) Use of tracers for soil and fertilizer nitrogen research. Adv Agron 28:219–266. https://doi. org/10.1016/S0065-2113(08)60556-8
- Haynes RJ (2005) Labile organic matter fractions as central components of the quality of agricultural soils: an overview. Academic Press, Adv Agron
- Hobbs PR, Sayre K, Gupta R (2008) The role of conservation agriculture in sustainable agriculture. Philos Trans R Soc Lond Ser B Biol Sci 363:543–555. https://doi.org/10.1098 /rstb.2007.2169

- Holland EA, Coleman DC (1987) Litter placement effects on microbial and organic-matter dynamics in an agroecosystem. Ecology 68:425–433. https://doi.org/10.2307/1939274
- Hood R, Merckx R, Jensen ES, Powlson D, Matijevic M, Hardarson G (2000) Estimating crop N uptake from organic residues using a new approach to the N-15 isotope dilution technique. Plant Soil 223:33–44. https://doi.org/10.1023 /A:1004789103949
- Hood R, van Kessel C, Vanlauwe B (2008) Use of tracer technology for the management of organic sources In: IAEA (ed) Guidelines on Nitrogen management in agricultural systems. . IAEA-TCS 29, Vienna, Austria
- Hopkinson JM, Walker B (1984) 21 seed production of Stylosanthes cultivars in Australia. In: Stace HM, Edye LA (eds) The biology and agronomy of Stylosanthes. Academic Press
- Husson O, Rakotondramanana A, Charpentier H, Michellon R, Ramaroson I, Razanamparany C, Andriantsilavo M, Moussa NLS (2006) An approach for creation, training and extension of systems based on direct seeding on permanent soil cover in Madagascar. In: Husson O, Rakotondralambo A (eds) In: Articles et posters présentés au troisième congrès mondial d'agriculture de conservation 2005. GSDM (Groupement Semis Direct de Madagascar), Nairobi, Kenya
- Jehne W (1984) 11 mycorrhizas and Stylosanthes. In: Stace HM, Edye LA (eds) The biology and agronomy of Stylosanthes. Academic Press
- Joergensen RG, Mueller T (1996) The fumigation-extraction method to estimate soil microbial biomass: calibration of the k(EN) value. Soil Biol Biochem 28:33–37. https://doi. org/10.1016/0038-0717(95)00101-8
- Jones A, Breuning-Madsen H, Brossard M, Dampha A, Deckers J, Dewitte O, Gallali T, Hallett S, Jones R, Kilasara M, Le Roux P, Micheli E, Montanarella L, Spaargaren O, Thiombiano L, Van Ranst E, Yemefack M, Zougmoré R (2013) Soil atlas of Africa. European comission, publications office of the european union, Luxembourg
- Kassim G, Martin JP, Haider K (1981) Incorporation of a wide variety of organic substrate carbons into soil biomass as estimated by the fumigation procedure. Soil Sci Soc Am J 45:1106–1112
- Koroleff F (1983) Determination of nutrients. In: Grasshoff K et al (eds) Methods of seawater analysis. Verlag Chemie, Weinheim
- Kull CA (2016) Bushfire in Madagascar: natural hazard, useful tool, and change agent. In: G Bankoff, Christensen J (eds) Natural hazards and peoples in the Indian Ocean world: bordering on danger. Palgrave Macmillan US, New York
- Lal R, Stewart BA (2014) Sustainable intensification of smallholder agriculture. CRC Press, Soil Management of Smallholder Agriculture
- Linder CR, Moore LA, Jackson RB (2000) A universal molecular method for identifying underground plant parts to species. Mol Ecol 9:1549–1559. https://doi.org/10.1046/J.1365-294 x.2000.01034.X
- Little DA, McIvor JG, McLean RW (1984) 19 the chemical composition and nutritive value of Stylosanthes. In: Stace HM, Edye LA (eds) The biology and agronomy of Stylosanthes. Academic Press
- Maltas A, Corbeels M, Scopel E, Wery J, da Silva FAM (2009) Cover crop and nitrogen effects on maize productivity in no-

tillage Systems of the Brazilian Cerrados. Agron J 101:1036– 1046. https://doi.org/10.2134/Agronj2009.0055

- Matos ED, Mendonca ED, Cardoso IM, de Lima PC, Freese D (2011) Decomposition and nutrient release of leguminous plants in coffee agroforestry systems. Rev Bras Cienc Solo 35:141–149
- Mayer J, Buegger F, Jensen ES, Schloter M, Hess J (2003) Estimating N rhizodeposition of grain legumes using a N-15 in situ stem labelling method. Soil Biol Biochem 35:21– 28. https://doi.org/10.1016/S0038-0717(02)00212-2
- McKeon GM, Mott JJ (1984) 16 seed biology of Stylosanthes. In: Stace HM, Edye LA (eds) The biology and agronomy of Stylosanthes. Academic Press
- McNeill AM, Fillery IRP (2008) Field measurement of lupin belowground nitrogen accumulation and recovery in the subsequent cereal-soil system in a semi-arid Mediterraneantype climate. Plant Soil 302:297–316. https://doi.org/10.1007 /S11104-007-9487-Y
- Médiène S, Valantin-Morison M, Sarthou J-P, de Tourdonnet S, Gosme M, Bertrand M, Roger-Estrade J, Aubertot J-N, Rusch A, Motisi N, Pelosi C, Doré T (2011) Agroecosystem management and biotic interactions: a review. Agron Sustain Dev 31:491–514. https://doi. org/10.1007/s13593-011-0009-1
- Mhlanga B, Cheesman S, Maasdorp B, Mupangwa W, Thierfelder C (2015) Contribution of cover crops to the productivity of maize-based conservation agriculture Systems in Zimbabwe. Crop Sci 55:1791–1805. https://doi.org/10.2135 /cropsci2014.11.0796
- Michellon R, Husson O, Moussa N, Randrianjafizanaka MT, Naudin K, Letourmy P, Andrianaivo A-P, Rakotondramanana, Raveloarijoana N, Enjalric F, Penot E, Séguy L (2011) Striga Asiatica: a driving-force for dissemination of conservation agriculture systems based on Stylosanthes Guianensis in Madagascar. World congress on conservation agriculture. 5, AUS
- Mommer L, Wagemaker CAM, De Karron H, Ouborg NJ (2008) Unravelling below-ground plant distributions: a real-time polymerase chain reaction method for quantifying species proportions in mixed root samples. Mol Ecol Resour 8: 947–953. https://doi.org/10.1111/j.1755-0998.2008.02130.x
- Mott JJ, Mckeon GM, Gardener CJ, Tmannetje L (1981) Geographic-Variation in the Reduction of Hard Seed Content of Stylosanthes Seeds in the Tropics and Subtropics of Northern Australia. Aust J Agr Res 32:861– 869. https://doi.org/10.1071/Ar9810861
- Muhr L, Tarawali SA, Peters M, Schultze-Kraft R (2002) Soil mineral N dynamics and maize grain yields following Centrosema Macrocarpum and Stylosanthes Guianensis: effects of different rotations and varying levels of N fertiliser. Field Crop Res 78:197–209. https://doi.org/10.1016/s0378-4290(02)00135-1
- Nan ZB, Hanson J, Yeshi WM (1998) Effects of sulphuric acid and hot water treatments on seedborne fungi and germination of Stylosanthes Hamata, S-guianensis and S-scabra. Seed Sci Technol 26:33–43
- Nguluu SN, Probert ME, McCown RL, Myers RJK, Waring SA (2001) Isotopic discrimination associated with symbiotic nitrogen fixation in stylo (Stylosanthes Hamata L.) and cowpea (Vigna Unguiculata L.) Nutr Cycl Agroecosyst 62:11–14. https://doi.org/10.1023/a:1015440906428

- Nyagumbo I, Mkuhlani S, Pisa C, Kamalongo D, Dias D, Mekuria M (2016) Maize yield effects of conservation agriculture based maize-legume cropping systems in contrasting agroecologies of Malawi and Mozambique. Nutr Cycl Agroecosyst 105:275–290. https://doi.org/10.1007/s10705-015-9733-2
- Oberson A, Frossard E, Buhlmann C, Mayer J, Mader P, Luscher A (2013) Nitrogen fixation and transfer in grass-clover leys under organic and conventional cropping systems. Plant Soil 371:237–255. https://doi.org/10.1007/S11104-013-1666-4
- Ojiem J, Vanlauwe B, de Ridder N, Giller K (2007) Niche-based assessment of contributions of legumes to the nitrogen economy of western Kenya smallholder farms. Plant Soil 292: 119–135. https://doi.org/10.1007/s11104-007-9207-7
- Orr DM (2010) Managing the grass-legume balance in Stylosanthes Scabra cv. Seca pastures in central Queensland. Trop Grasslands 44:174–183
- Peel MC, Finlayson BL, McMahon TA (2007) Updated world map of the Koppen-Geiger climate classification. Hydrol Earth Syst Sci 11:1633–1644
- Peoples MB, Bowman AM, Gault RR, Herridge DF, McCallum MH, McCormick KM, Norton RM, Rochester IJ, Scammell GJ, Schwenke GD (2001) Factors regulating the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. Plant Soil 228: 29–41. https://doi.org/10.1023/a:1004799703040
- Probert ME (1984) 10 the mineral nutrition of Stylosanthes. In: Stace HM, Edye LA (eds) The biology and agronomy of Stylosanthes. Academic Press
- Rasche F, Cadisch G (2013) The molecular microbial perspective of organic matter turnover and nutrient cycling in tropical agroecosystems - what do we know. Biol Fert Soils 49:251– 262. https://doi.org/10.1007/S00374-013-0775-9
- R-Core-Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Rusinamhodzi L, Corbeels M, van Wijk MT, Rufino MC, Nyamangara J, Giller KE (2011) A meta-analysis of longterm effects of conservation agriculture on maize grain yield under rain-fed conditions. Agron Sustain Dev 31:657–673. https://doi.org/10.1007/S13593-011-0040-2
- Saito K, Azoma K, Oikeh SO (2010) Combined effects of Stylosanthes Guianensis fallow and tillage management on upland rice yield, weeds and soils in southern Benin. Soil Tillage Res 107:57–63. https://doi.org/10.1016/j. still.2010.03.001
- Salas AM, Elliott ET, Westfall DG, Cole CV, Six J (2003) The role of particulate organic matter in phosphorus cycling. Soil Sci Soc Am J 67:181–189
- Shearer G, Kohl DH (1986) N-2-fixation in field settings estimations based on natural N-15 abundance. Aust J Plant Physiol 13:699–756
- Shelton HM, Humphreys LR (1975) Undersowing rice (oryzasativa) with stylosanthes-guyanensis .2. delayed sowing time and crop variety. Exp Agric 11:97–101. https://doi. org/10.1017/S0014479700006530
- Six J, Feller C, Denef K, Ogle SM, Moraes Sa JC, Albrecht A (2002) Soil organic matter, biota and aggregation in temperate and tropical soils - effects of no-tillage. Agronomie 22: 755–775

- Six J, Ogle SM, Jay breidt F, Conant RT, Mosier AR, Paustian K (2004) The potential to mitigate global warming with notillage management is only realized when practised in the long term. Glob Chang Biol 10:155–160. https://doi. org/10.1111/j.1529-8817.2003.00730.x
- Szott LT, Palm CA, Davey CB (1994) Biomass and litter accumulation under managed and natural tropical fallows. For Ecol Manag 67:177–190. https://doi.org/10.1016/0378-1127(94) 90015-9
- Thierfelder C, Wall PC (2011) Reducing the risk of crop failure for smallholder farmers in Africa through the adoption of conservation agriculture. In: Bationo A, Waswa B, Okeyo JMM, Maina F, Kihara JM (eds) Innovations as key to the green revolution in Africa. Springer, Netherlands, pp 1269–1277
- Thierfelder C, Cheesman S, Rusinamhodzi L (2013) Benefits and challenges of crop rotations in maize-based conservation agriculture (CA) cropping systems of southern Africa. Int J Agric Sustain 11:108–124. https://doi.org/10.1080 /14735903.2012.703894
- Thomas RJ, Asakawa NM (1993) Decomposition of leaf litter from tropical forage grasses and legumes. Soil Biol Biochem 25:1351–1361. https://doi.org/10.1016/0038-0717 (93)90050-1
- Thomas D, Sumberg JE (1995) A review of the evaluation and use of tropical forage legumes in sub-Saharan Africa. Agric Ecosyst Environ 54:151–163. https://doi.org/10.1016/0167-8809(95)00584-f
- Tian G, Kang BT (1998) Effects of soil fertility and fertilizer application on biomass and chemical compositions of leguminous cover crops. Nutr Cycl Agroecosyst 51:231–238. https://doi.org/10.1023/A:1009785905386
- Unkovich MJ, Pate JS (2000) An appraisal of recent field measurements of symbiotic N-2 fixation by annual legumes. Field Crop Res 65:211–228. https://doi.org/10.1016/S0378-4290(99)00088-X
- Unkovich M, Herridge D, Peoples M, Cadisch G, Boddey B, Giller K (2008) Measuring plant-associated nitrogen fixation in agricultural systems
- Unkovich MJ, Baldock J, Peoples MB (2010) Prospects and problems of simple linear models for estimating symbiotic N-2 fixation by crop and pasture legumes. Plant Soil 329:75– 89. https://doi.org/10.1007/s11104-009-0136-5
- Urquiaga S, Cadisch G, Alves BJR, Boddey RM, Giller KE (1998) Influence of decomposition of roots of tropical forage species on the availability of soil nitrogen. Soil Biol Biochem 30: 2099–2106. https://doi.org/10.1016/s0038-0717(98)00086-8
- Vallis I, Gardener CJ (1984) 18 nitrogen inputs into agricultural systems by Stylosanthes. In: Stace HM, Edye LA (eds) The biology and agronomy of Stylosanthes. Academic Press
- van Kessel C, Clough T, van Groenigen JW (2009) Dissolved organic nitrogen: an overlooked pathway of nitrogen loss from agricultural systems. J Environ Qual 38:393–401. https://doi.org/10.2134/Jeq2008.0277
- Vance ED, Brookes PC, Jenkinson DS (1987) Microbial biomass measurements in Forest soils - the use of the chloroform fumigation incubation method in strongly acid soils. Soil Biol Biochem 19:697–702. https://doi.org/10.1016/0038-0717(87)90051-4
- Vanlauwe B, Sanginga N, Merckx R (1998) Recovery of Leucaena and Dactyladenia residue Nitrogen-15 in alley cropping

systems. Soil Sci Soc Am J 62:454–460. https://doi. org/10.2136/sssaj1998.03615995006200020023x

- Wilcox R (2012) Introduction to robust estimation and hypothesis testing. Academic Press, San Diego
- WMO (2015) Climate in Africa: 2013. World Meteorological Organization (WMO), no. 1147, Geneva, Switzerland
- WRB IWG (2014) World reference base for soil resources. International soil classification system for naming soils and creating legends for soil maps. World soil resources reports, No. 106. FAO, Rome
- Yoneyama T, Fujita K, Yoshida T, Matsumoto T, Kambayashi I, Yazaki J (1986) Variation in natural abundance of N-15 among plant-parts and in N-15-N-14 fractionation during

N-2 fixation in the legume-rhizobia symbiotic system. Plant Cell Physiol 27:791–799

- Yoshida S (1981) Fundamentals of rice crop science. International Rice Research Institute, Los Banos, Philippines
- Yuste JC, Penuelas J, Estiarte M, Garcia-Mas J, Mattana S, Ogaya R, Pujol M, Sardans J (2011) Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Global Change Biol 17:1475–1486. https://doi. org/10.1111/J.1365-2486.2010.02300.X
- Zemek O (2016) In transition to conservation agriculture: nitrogen dynamics in a rice - Stylosanthes guinanesis crop rotation in the Midwest highlands of Madagascar. D-USYS, ETH, Zurich