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Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses

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Summary

1. Efficient resource use is a key factor for sustainable production and a necessity for meeting future global food demands. However, the factors that control resource use efficiency in agro-ecosystems are only partly understood.

2. We investigated the influence of soil biota on nutrient leaching, nutrient-use efficiency and plant performance in outdoor, open-top lysimeters comprising a volume of 230 L. The lysimeters were filled with sterilized soil in two horizons and inoculated with a reduced soil-life inoculum (soil biota ≤11 μm, microbially dominated) and an enriched soil-life inoculum [soil organisms ≤2 mm, also containing arbuscular mycorrhizal fungi (AMF)]. A crop rotation was planted, and nutrient leaching losses, plant biomass and nutrient contents were assessed over a period of almost 2 years.

3. In the first year of the experiment, enriched soil life increased crop yield (+22%), N uptake (+29%) and P uptake (+110%) of maize and strongly reduced leaching losses of N (−51%, corresponding to a reduction of 76 kg N ha^{-1}). In the second year, wheat biomass (+17%) and P contents (+80%) were significantly increased by enriched soil life, but the differences were lower than in the first year.

4. Enriched soil life also increased P mobilization from soil (+112%) and significantly reduced relative P leaching losses (−25%), defined as g P leached per kg P plant uptake, as well as relative N leaching losses (−36%), defined as kg N leached per kg N plant uptake, demonstrating that nutrient-use efficiency was increased in the enriched soil-life treatment.

5. *Synthesis and applications*. Soil biota are a key factor determining resource efficiency in agriculture. The results suggest that applying farming practices, which favour a rich and abundant soil life (e.g. reduced tillage, organic farming, crop rotation), can reduce environmental impacts, enhance crop yield and result in a more sustainable agricultural system. However, this needs to be confirmed in field situations. Enhanced nutrient-use efficiency obtained through farming practices which exert positive effects on soil biota could result in reduced amounts of fertilisers needed for agricultural production and reduced nutrient losses to the environment, providing benefits of such practices beyond positive effects on biodiversity.

Key-words: agro-ecosystem, arbuscular mycorrhizal fungi, crop rotation, lysimeters, maize, nutrient losses, nutrient-use efficiency, phosphorous, tillage, wheat

Introduction

The high agricultural yields currently produced in many parts of the world are often achieved with the aid of excessive fertilizer use (Ju *et al*. 2009). Only about 50% of

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N inputs to agricultural lands are used by crops, with a huge fraction of the inputs remaining unused for agricultural purposes and being lost through leaching and gas emissions (Smil 1999; Liu *et al*. 2010). These nutrient losses are known to cause severe environmental problems like ground- and surface-water pollution and eutrophication, reduced biodiversity in ecosystems and to contribute to global warming (Galloway *et al*. 2003; Schlesinger 2009). Moreover, these nutrients represent valuable resources which might become limited in the near future. For instance, the globally available stocks of phosphate are expected to be depleted in the next 50–100 years (Cordell, Drangert & White 2009). There is an urgent need to change paradigms towards sustainable agricultural practices that aim to use applied resources as efficiently as possible to ensure sufficient yields and reduce environmental impacts (Schlesinger 2009).

Most nutrient transformations in soil are performed by soil organisms. Through their activities, they drive nutrient cycling and play an important role in determining whether nutrients are made available to plants, are stored in the soil or are prone to being lost from the plant–soil system (Robertson & Groffman 2007; van der Heijden, Bardgett & van Straalen 2008). Several studies have addressed the importance of soil biota and their interactions for nutrient mineralization and plant nutrition (e.g. Ingham *et al*. 1985; Setälä & Huhta 1991). Commonly, nutrient mineralization and plant nutrition are increased by faunal activities, but increased nutrient loss through leaching is also often reported (Setälä *et al*. 1990; Bardgett & Chan 1999).

Particular attention is given to arbuscular mycorrhizal fungi (AMF), a group of soil fungi that live in symbiosis with the majority of land plants, including many agricultural crops. AMF can mobilize nutrients from soil, transfer them to their host plants and improve plant nutrition (Smith & Read 2008). Exploration of a larger soil volume and efficient nutrient uptake are considered key mechanisms for the improvement of plant nutrition through AMF (Jakobsen, Abbott & Robson 1992). Many studies observed a positive effect of AMF on P nutrition. In contrast, the contribution of AMF to plant N nutrition is far less clear (Smith & Smith 2011). It was shown that these fungi can reduce losses of P (Asghari *et al*. 2005; van der Heijden 2010) and N (Asghari & Cavagnaro 2012) through leaching. Enhanced nutrient interception of AMF rooting systems is considered a main mechanism for the reduction of nutrient leaching losses.

Interactions between soil fauna and AMF were reported to result in positive effects on plant biomass (Klironomos & Kendrick 1995; Gange 2000) and sometimes enhanced plant nutrition (Lussenhop 1996). A synergistic effect of enhanced mineralization by soil fauna with enhanced nutrient interception by AMF rooting systems, as indicated by a study of Koller *et al*. (2013), could result in a highly efficient nutrient cycling machinery that enhances nutrient mobilization from soil resources and provides an effective uptake pathway of the mobilized nutrients to plants. If applied to agriculture, this effect would enhance agricultural sustainability by promoting internal nutrient cycling and reducing the need for external nutrient inputs. However, little is known about such interactive effects on nutrient cycling, plant nutrition and especially nutrient losses under ecologically relevant conditions (e.g. field settings). The majority of investigations addressing these issues were conducted in small microcosms in the greenhouse with questionable ecological relevance and transferability to field situations (Kampichler, Bruckner & Kandeler 2001; Read 2002). The investigation of the effects of soil biota and their food webs on nutrient cycling in field settings is difficult, because removing soil biota to obtain adequate control treatments is rarely possible without strong perturbations of the whole soil ecosystem (Hunt *et al*. 1987).

Here, we assess the impact of soil biota on plant growth, nutrient-use efficiency and nutrient losses in outdoor lysimeters. To enhance the scale of the experiment and improve ecological relevance, we used outdoor lysimeters comprising a volume of 230 L. The lysimeters were filled with top- and subsoil to imitate the natural soil profile. This approach provides a clear advantage to studies in much smaller microcosms, as side effects like root and hyphal growth constraints due to limited soil volume are reduced. We planted an agricultural crop rotation in lysimeters either inoculated with an enriched soil-life inoculum (soil organisms $\leq$2 mm, including AMF) or a reduced soil-life inoculum (soil biota $\leq$ 11 μm, microbially dominated). This enabled us to test whether soil food web complexity influences nutrient cycling (see Methods for further information).

Plant nutrition, biomass and grain yield, and leaching losses of soil nutrients were monitored over a period of almost 2 years. We hypothesized that enriched soil life (i) increases plant biomass, nutrient contents and crop yields and (ii) reduces the leaching losses of soil nutrients. Our study demonstrates that soil biota contribute substantially to agricultural sustainability by supporting plant nutrient uptake and plant yield and by reducing nitrogen leaching losses.

Materials and methods

LYSIMETER SET-UP

The outdoor lysimeter facility used here was established in 1971 and consists of 32 lysimeters each with an inner diameter of 59 cm and a depth of 84 cm, resulting in a volume of *c*. 230 L. The lysimeters consist of a polypropylene container inserted into a concrete body. A hole in the bottom of the container collects soil water drainage that is stored in 25-L plastic containers positioned in a closed cabinet under the lysimeter (Figs 1 and S1 in Supporting information). Before the start of the experiment, 16 lysimeters were emptied, cleaned and sterilized by spraying with a 1·2% active chlorine bleach solution and rinsing with water.

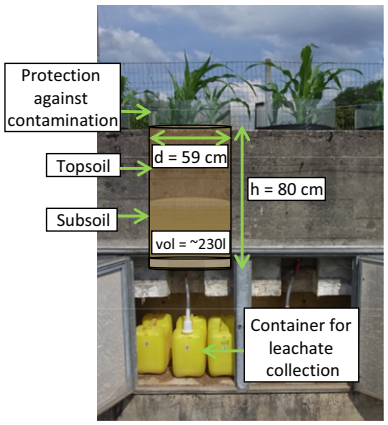

Fig. 1. Set-up of the lysimeters.

Six cubic metres of a soil classified as *calcaric cambisol* was collected from a long-term pasture on an organic farm near the Agroscope Research Station in Zürich, Switzerland (47°43′11·83″ N, 8°53′65·25″ E). The pasture has had manure regularly applied. Top- (0–30 cm) and subsoil (30–80 cm) were collected separately and processed and sterilized as described in supporting methods (Appendix S1, Supporting information).

INOCULUM PRODUCTION

Two soil inocula were produced in the greenhouse by combining a sterile sand–soil mixture with either fresh or autoclaved field soil (both passed through a 2-mm sieve). This soil was also used to fill the lysimeters. Both inocula received a microbial wash (soil suspension, 11 μm filtered, see Appendix S1, Supporting information for details). Pots were planted, grown in the greenhouse for 4 months, air-dried and harvested (see Appendix S1, Supporting information for details). The substrate in the pots inoculated with fresh field soil, including cut root pieces, was used to set up the enriched soil-life (ENR) treatment containing soil organisms ≤2 mm (e.g. members of the soil meso- and microfauna, AMF and microflora). The substrate with cut root pieces produced from the autoclaved field soil was used to set up the reduced soil-life (RED-) treatment containing predominantly micro-organisms ≤11 μm and some protozoa. The abundance of various soil biota present in both inocula before filling lysimeters was conducted by Earthfort Testing Services (Corvallis, OR, USA) and is shown in Table 1. Thirteen litres of inoculum were added to each lysimeter.

We focused on soil organisms ≤2 mm as larger soil organisms, such as earthworms, are present in lower numbers in soil and may not have been distributed equally among the different inoculum pots. The size fraction of organisms ≤11 μm for the RED treatment was chosen to provide a microbial community providing basic ecosystem functions, but to exclude AMF. Moreover, a range of studies showed that larger soil biota (e.g. those bigger than 11 μm), including AMF, are more negatively affected by intensive agricultural management (e.g. intensive ploughing, reduced crop diversity and heavy fertilization) compared to smaller sized organisms such as bacteria and fungi (Wardle 1995; Bradley, Drijber & Knops 2006; Postma-Blaauw *et al.* 2010). The cultivation step in the greenhouse in nutrient-poor substrate provided conditions favourable for the propagation of AMF in the ENR treatment and served to establish a well-developed microbial community in both inocula. Despite the addition of a microbial wash, we cannot rule out the possibility that the microbial communities differed between the inoculums, as the presence or absence of other groups of soil organisms is likely to affect microbial communities.

Table 1. The abundance of bacteria, fungi and protozoa in the inoculum used to create the treatments as determined from a composite sample of all inoculum pots by Earthfort Testing Services. AMF root colonization was determined from three subsamples of a composite sample. Active bacteria and fungi were quantified by microscopy after staining with fluorescin diacetate. Total bacteria were quantified by microscopy using a fluorescein isothiocyanate method. Fungal biovolume was measured under the microscope and converted into total fungal biomass. Protozoa were quantified with a most probable number approach after direct counting. Nematodes were counted by microscopy. AMF root colonization was assessed as described in Appendix S1 (Supporting information)

	Enriched soil life	Reduced soil life
Micro-organisms (μg g^{-1})		
Active bacteria	5·06	4·03
Total bacteria	434	343
Active fungi	0	0
Total fungi	39·9	44·4
Hyphal diameter (μm)	2·6	2·7
Protozoa (no g^{-1})		
Flagellates	8356	5785
Amobae	13 927	5785
Ciliates	0	28
Nematodes (no g^{-1})	0·02	0
AMF root colonization (%) (n = 3)		
Total	59·67 (6·17)	0 (0·00)
Vesicles	13·33 (2·91)	0 (0·00)
Arbuscles	15·33 (4·63)	0 (0·00)

LYSIMETER FILLING

The lysimeters were filled as described in Appendix S1 (Supporting information). The characteristics of the subsoil and the topsoil–inoculum mixture filled into the lysimeters are presented in Table 2. The experiment was arranged in two blocks and comprised of two treatments each replicated eight times.

PLANTING

All seeds were surface-sterilized before planting by stirring in 1·25% bleach for 10 min. The crop rotation started with maize and was planted in May 2011. In July 2011, a grass mixture was sown between the maize plants to provide AMF host plants over the winter. Maize was harvested in August 2011 and the grass mixture in March 2012. Subsequently, summer wheat was sown and harvested 4 months later in July 2012. Finally, a grass–clover mixture was grown until January 2013. Details are given in Appendix S1 (Supporting information).

FERTILIZATION AND WATERING

We expected considerable nutrient mobilization from the soil sterilization process and therefore did not fertilize in the first year. In the second year, wheat was fertilized with commercial NH_4NO_3

Table 2. Characteristics of the sterilized subsoil and topsoil used to fill the lysimeters

	Topsoil	Subsoil
Clay (%)	25·05	26·50
Silt (%)	33·25	55·90
Sand (%)	38·70	17·30
Humus (%)	3·00	0·30
Organic C (t ha^{-1})	102·01	11·04
N total (t ha^{-1})	12·79	2·30
Available P (kg ha^{-1})	5·331	0·358
P_{AAE} (kg ha^{-1})	128·17	13·25
K_{AAE} (t ha^{-1})	0·80	0·60
Mg_{AAE} (t ha^{-1})	9·9	66·0
Ca_{AAE} (t ha^{-1})	137·6	306·0
$CaCO_3$ (t ha^{-1})	976·54	3069·12
pH (H_2O)	7·50	8·60

fertilizer corresponding to 50 kg N ha^{-1} applied on 16 April 2012 and 30 kg N ha^{-1} on 23 May 2012. Lysimeters were watered on three occasions between July and September in 2011 (a total of 25 L per lysimeter) with tap water when maize plants were water-stressed because of summer drought and turgor loss was visible.

SAMPLING

Sampling of leachates, roots for determining AMF colonization, soil for microbial biomass C and N contents and of plant biomass was conducted as described in Appendix S1 (Supporting information).

LEACHATE ANALYSES

Concentrations of NO_3–N, NO_2–N, PO_4–P and SO_4 were determined by anion chromatography, and total P was determined photometrically after oxidation. NH_4 concentration was determined by continuous flow analysis, and total dissolved N (TDN) was measured by chemoluminescence (see Appendix S1 for details, Supporting information). All nutrient concentrations were multiplied with the leachate volume to calculate the total amount of nutrients lost per lysimeter.

The difference between TDN and mineral N (NO_3–N, NO_2–N and NH_4–N) was considered as dissolved organic N (DON). The amount of PO_4–P in the leachates was labelled reactive P. The difference between total P and reactive P was labelled unreactive P. This fraction comprises all compounds not directly available to plants such as soluble and particulate organic P compounds, polyphosphates and particulate inorganic material, for example clays (Pote, Daniel & DeLaune 2009).

Leaching data are presented on a yearly basis with year 1 ranging from maize sowing in May 2011 until harvest of the grass mixture in March 2012 and year 2 ranging from wheat sowing in March 2012 to grass–clover harvest in January 2013.

PLANT NUTRIENT CONCENTRATIONS

The dried plant material was weighed, and P concentrations were analysed photometrically according to Watanabe & Olsen (1965) after dry ashing. N concentrations were analysed after combustion with an elemental analyser (varioMax CN; Elementar, Hanau, Germany).

SOIL BIOLOGICAL PARAMETERS

Arbuscular mycorrhizal fungi root infection was quantified after staining using a modified grid-line intersection method on 100 intersections per sample (McGonigle *et al*. 1990). Soil microbial biomass C and N contents were analysed with chloroform fumigation extraction as described in Appendix S1 (Supporting information).

SOIL ANALYSES

Soil texture, organic C, humus, $CaCO_3$, soil pH, available soil P extracted with CO_2-saturated water, ammonium acetate–EDTA-extractable soil P (PAAE), K, Mg, K, Ca and Mg and total soil N were all analysed using standard methods according to the reference protocols of the Swiss Federal Research Stations (Eidgenössische Forschungsanstalten FAL 1996). Soil analyses at the end of the experiment were performed in the topsoil only.

For the whole experimental period, we calculated the amount of soil P that had been mobilized from initial non-AAE-extractable soil P resources (P_{mob}) as

$$P_{mob} = (P_{plant} + P_{leached}) - (PAAE_{start} - PAAE_{end}),$$

where P_{plant} is the amount of P in total plant biomass, $P_{leached}$ is the total amount of P leached during the whole experiment, $PAAE_{start}$ is the amount of PAAE at the start of the experiment and $PAAE_{end}$ is the amount of PAAE at the end of the experiment.

STATISTICAL ANALYSES

To test if the inoculation treatments had any overall effect on nutrient cycling and plant growth, a multivariate analysis of variance (MANOVA) was conducted for the complete experiment and for years 1 and 2 separately. The models included Block and Treatment as factors and total plant biomass, plant N contents and P contents, total N leaching and P leaching and SO_4-leaching as dependent variables. Where the MANOVA rendered significant treatment effects, two-way ANOVAs, including the block effect and the inoculation treatment as factors were performed for all measured plant and nutrient compounds to allow biological interpretation of the results. Model residuals were checked for normality and homoscedasticity by plotting fitted values against residuals, and data were log-transformed where necessary.

Three multiple regression models with the explanatory variables Block, Treatment and average AMF root colonization during the whole experiment were fitted to assess the contribution of the inoculation treatments and AMF root colonization to the observed effects on plant N uptake and P uptake and biomass and to allow inferences about the mechanisms behind our results. All analyses were performed with the R-statistical software, version 3.0.1 (R Core Team 2013).

Results

OVERALL EFFECT

The MANOVA showed strong treatment effects on plant performance and nutrient leaching for the whole experiment,

as well as for year 1 and 2 separately (Table S4, Supporting information). In the following sections, we present the results of univariate two-way ANOVAS for all measured variables for the different years and the whole experiment.

Year 1

Leaching. Leaching losses of NO_3–N, DON and of total dissolved N were significantly reduced in the ENR treatment compared to the RED treatment (Fig. 2, Table S2, Supporting information). In the first year, total N leached was 74·4 kg ha^{-1} for the ENR treatment and 150·6 kg ha^{-1} for the RED treatment. This is a reduction of total N leaching by 51·5% corresponding to an amount of 76·2 kg N ha^{-1}. Approximately 86% of total N leaching occurred in the form of NO_3–N, and on average, 13·5% was in dissolved organic form (Fig. 2, Table S2, Supporting information).

In contrast, total P and reactive P leaching was significantly higher in the ENR treatment, with total P leached amounting to 0·2 kg ha^{-1} for the ENR treatment and 0·12 kg ha^{-1} for the RED treatment. Leaching of unreactive P was significantly reduced in the ENR treatment (Fig. 3, Table S2, Supporting information). SO_4 leaching was also significantly reduced in the ENR treatment, amounting to 108·7 kg ha^{-1} in the ENR treatment and 128·1 kg ha^{-1} in the RED treatment (Table S2, Supporting information).

Crop performance. Maize yield was 40·3 t ha^{-1} in the ENR treatment and 33·2 t ha^{-1} in the RED treatment, corresponding to an increase of 22·3% in the ENR treatment (Fig. 4a). ENR plants also contained 28·9% more N and 110% more P than RED plants (Fig. 4b; Table S2, Supporting information). The grass mixture grown as intercrop under the maize performed slightly better in the RED treatment compared to the ENR treatment, but its biomass was much lower than the maize biomass (Table S2, Supporting information).

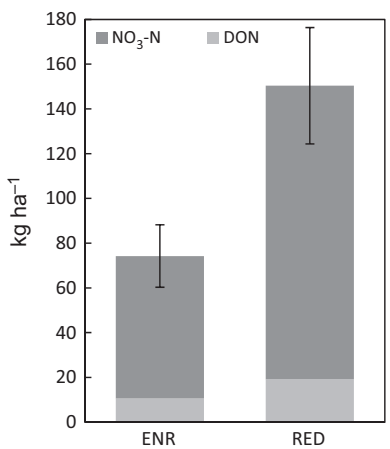

Fig. 2. Leaching losses of DON and NO_3-N in lysimeters inoculated with an enriched (ENR) or reduced (RED) soil-life inoculum in year 1 of the experiment. Error bars show ± 1 standard error of total N leaching losses (n = 8). Note that NH_4 leaching was very low compared to the other N compounds leached (soil biota: 0·18 kg NH_4-N ha^{-1}, control: 0·21 kg NH_4-N ha^{-1}), and is, hence, not displayed here. DON, dissolved organic N.

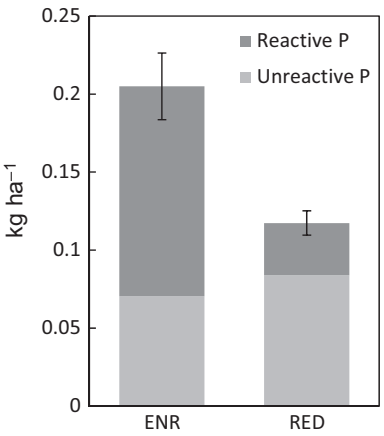

Fig. 3. Leaching losses of different P fractions from lysimeters inoculated with an enriched (ENR) or reduced (RED) soil-life inoculum in year 1 of the experiment. Error bars show ± 1 standard error of total P leaching losses (n = 8).

Year 2

Leaching. In the second year, the differences in leaching losses between the treatments were smaller and not significant. Total N leached averaged 109·9 kg ha^{-1} in the ENR treatment and 93·0 kg ha^{-1} in the RED treatment. Total P leached amounted to 0·3 and 0·27 kg ha^{-1} in the ENR treatment and RED treatment, respectively. Leaching of unreactive P was significantly reduced in the ENR treatment, compared to the RED treatment. The amount of SO_4 leached was lower than in the first year and was significantly reduced in the ENR treatment (Table 3).

Crop performance. Total wheat biomass, P content and P concentration were significantly increased in the ENR treatment compared to the RED treatment, with only a slight increase in N content (Table 3). Wheat yield did not differ significantly between the treatments, but P concentration in the grains was significantly increased by 33·3% in the ENR treatment. Biomass of weeds growing with the wheat was also higher in the ENR treatment compared to the RED treatment (Table 3). In the grass–clover mixture sown after wheat, no difference in plant biomass between the treatments was detected (Table 3).

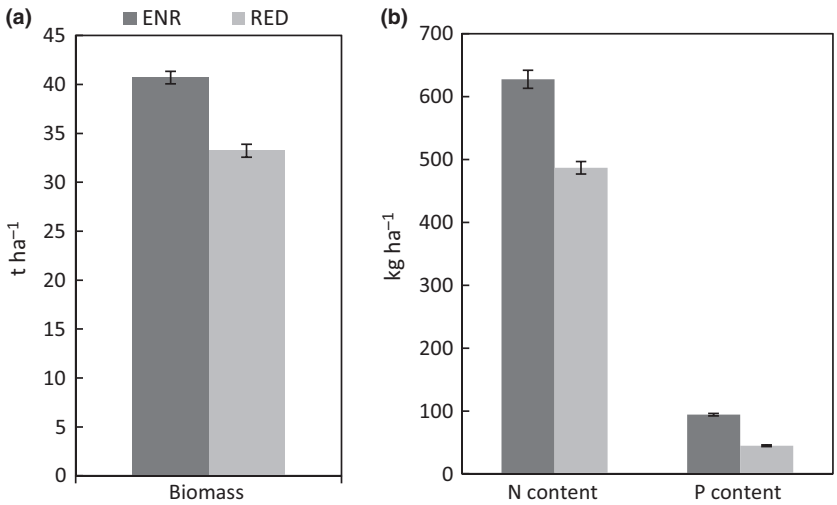

Fig. 4. Maize biomass (a) and nutrient contents (b) of plants grown in lysimeters inoculated with an enriched (ENR, dark grey) or reduced soil-life inoculum (RED, light grey). Error bars show ± 1 standard error (n = 8).

CUMULATIVE NUTRIENT LOSS AND PLANT UPTAKE FOR THE WHOLE EXPERIMENT

The cumulative amount of N lost through leaching was 184·3 kg ha^{-1} in the ENR treatment and 243·6 kg ha^{-1} in the RED treatment. This is a reduction of 24·3% in the ENR treatment, corresponding to 59·3 kg N ha^{-1} (Fig. 5a). Total N in plant biomass was 879·9 kg N ha^{-1} in the ENR treatment and 747·2 kg N ha^{-1} in the RED treatment. This is an increase of 17·8% in the ENR treatment, corresponding to 132·7 kg N ha^{-1} (Fig. 5a). Over the whole experiment, relative N leaching, defined as kg N leached per kg N plant uptake, amounted to 0·21 kg N in the ENR treatment and 0·33 kg N in the RED treatment (Table S3, Supporting information). Total P leached amounted to 0·51 kg ha^{-1} and 0·39 kg ha^{-1} in the ENR treatment and RED treatment, respectively. This is an increase of 0·12 kg ha^{-1} in the ENR treatment (Fig. 5b). The fraction of unreactive P leaching was, however, significantly reduced in the ENR treatment (Table S3, Supporting information).

Total P in plant biomass amounted to 121·8 kg ha^{-1} and 70·7 kg ha^{-1} in the ENR treatment and RED treatment, respectively. This is an increase of 72·3%, corresponding to 51·1 kg P ha^{-1} (Fig. 5b). Relative P leaching (g P leached/kg P plant uptake) was lower in the ENR treatment and amounted to 4·13 g P, while in the RED treatment, relative P leaching was 5·47 g P (Table S3, Supporting information). 28% of P lost was in the form of unreactive P.

Total leaching losses of SO_4 amounted to 171·5 kg ha^{-1} and 204·2 kg ha^{-1} in the ENR treatment and RED treatment, respectively. This is a reduction of 16% in the ENR treatment, corresponding to 32·7 kg ha^{-1} (Table S3, Supporting information). The total amount of leachate did not differ significantly between the treatments (P = 0·08).

AMF ROOT COLONIZATION AND MICROBIAL BIOMASS

Four months after the start of the experiment, roots of the RED treatment already showed 10·25% root colonization by AMF, while the ENR treatment showed 75% root colonization (Table 4). The AMF root colonization of wheat was higher compared to maize in both treatments. The ENR treatment had 88·6% of root length colonized, while the RED treatment had 28·1% root length colonized. In the grass–clover mixture, the root colonization in the ENR treatment increased further, and the difference between the treatments decreased, although remaining significant (Table 4).

Microbial biomass C and N contents did not differ significantly between the treatments in both years (Table 4).

CONTRIBUTION OF AMF ROOT COLONIZATION TO PLANT N UPTAKE AND P UPTAKE

The multiple regression models containing Block, Treatment and AMF root colonization as predictors explained 70% of plant biomass, 66% of plant N uptake and 97% of plant P uptake. AMF root colonization was a significant predictor of plant P uptake, but not of plant N uptake and biomass. The inoculation treatment was a significant predictor of plant P uptake and plant biomass but not of plant N uptake (Table S5, Supporting information).

SOIL ANALYSES

At the end of the experiment, available soil P extracted with CO_2-saturated water was significantly reduced by 39·5% in the ENR treatment compared to the RED treatment. For PAAE, similar results were found. However, all other soil parameters did not differ significantly between the treatments. P export from soil through plant

Table 3. Leaching losses, plant biomass, nutrient contents and the respective ANOVA results for lysimeters inoculated with an enriched (ENR) or reduced (RED) soil-life inoculum in year 2. Means are shown ± 1 standard error (n = 8)

	ENR	RED	d.f.	*F*-value	*P*-value
Leaching losses					
NO_3–N (kg ha^{-1})	96·48 (9·47)	78·7 (5·94)	1,13	2·44	0·142
NH_4–N (kg ha^{-1})	0·65 (0·01)	0·71 (0·02)	1,13	4·12	0·063
DON (kg ha^{-1})	12·72 (0·89)	13·52 (0·37)	1,13	0·68	0·426
TDN (kg ha^{-1})	109·9 (10·24)	93·0 (6·24)	1,13	1·93	0·189
Reactive P (kg ha^{-1})	0·23 (0·02)	0·19 (0·03)	1,13	1·29	0·276
Unreactive P (kg ha^{-1})	0·07 (0·00)	0·08 (0·00)	1,13	6·63	0·023
Total P (kg ha^{-1})	0·30 (0·02)	0·27 (0·03)	1,13	0·55	0·473
SO_4 (kg ha^{-1})	62·8 (3·05)	76·1 (3·17)	1,13	8·44	0·012
Plant biomass and nutrient data					
Wheat					
Biomass					
Total (t ha^{-1})	10·6 (0·20)	9·1 (0·52)	1,13	7·22	0·019
N content (kg ha^{-1})	154·1 (4·58)	140·5 (5·93)	1,13	3·2	0·098
P content (kg ha^{-1})	35·96 (1·16)	19·9 (2·26)	1,13	37·0	0·000
N concentration (mg kg DW^{-1})	14·52 (0·30)	15·65 (0·64)	1,13	2·75	0·121
P concentration (mg kg DW^{-1})	3·38 (0·06)	2·24 (0·28)	1,13	15·1	0·002
N/P ratio*	4·29 (0·07)	7·57 (0·72)	1,13	27·2	0·000
Yield (kernels)					
Total (t ha^{-1})	3·40 (0·08)	3·50 (0·07)	1,13	2·93	0·110
N content (kg ha^{-1})	104·5 (2·26)	106·4 (2·45)	1,13	0·32	0·580
P content (kg ha^{-1})	20·29 (0·65)	15·79 (1·40)	1,13	8·44	0·012
N concentration (mg kg DW^{-1})	31·04 (0·36)	30·47 (0·71)	1,13	0·75	0·402
P concentration (mg kg DW^{-1})	6·02 (0·09)	4·51 (0·38)	1,13	13·81	0·003
Weed biomass	0·49 (0·13)	0·19 (0·04)	1,13	4·67	0·050
Grass–clover					
Total (t ha^{-1})	1·27 (0·25)	1·17 (0·12)	1,13	0·14	0·711
N content (kg ha^{-1})	35·86 (5·47)	32·6 (2·73)	1,13	0·30	0·596
P content (kg ha^{-1})	4·44 (0·92)	3·77 (0·41)	1,13	0·48	0·501
N concentration (mg kg DW^{-1})	29·82 (1·30)	28·11 (0·74)	1,13	1·51	0·241
P concentration (mg kg DW^{-1})	3·38 (0·15)	3·2 (0·07)	1,13	1·34	0·267
N/P ratio	8·98 (0·65)	8·81 (0·35)	1,13	0·07	0·790

DON, Dissolved organic N; TDN, total dissolved N.
*Log transformed.

uptake and leaching was higher than the decline in PAAE during the course of the experiment. We calculated the difference between P removed from the AAE-extractable soil P pool and P exported through plant uptake and leaching (P_{mob}). This gives an indication of how much soil P was mobilized from non-AAE-extractable soil pools during the course of the experiment. In the ENR treatment, an additional 62·8 kg P ha^{-1} had been mobilized, while in the RED treatment, only an additional 30·5 kg P ha^{-1} been mobilized from soil. Therefore, the presence of soil biota increased P mobilization from initially nonavailable soil P resources by 118% (Table 5).

Discussion

Our study demonstrates that soil life contributes substantially to agricultural sustainability by supporting plant nutrient uptake and plant yield and by reducing N leaching losses. This also indicates that soil life enhanced nutrient-use efficiency in our cropping system. Throughout the experiment, N leaching was reduced, while plant N and P contents were strongly increased in lysimeters with enriched soil life compared to lysimeters with a reduced soil-life treatment. To our knowledge, this is the first study showing that soil biota ranging between 11 μm and 2 mm, including AMF and the soil micro-and mesofauna, can exert strong effects on nutrient leaching and crop performance in an agricultural crop rotation.

Our observation is important because large amounts of N are lost through leaching from croplands (Liu *et al.* 2010). For instance, in Western European countries, estimated N leaching losses range from 5 to 102 kg N ha^{-1}a^{-1} (Leip *et al.* 2008), with losses up to 160 kg N ha^{-1}a^{-1} being reported (Herzog *et al.* 2008). Commonly, the highest leaching losses occur in areas under intensive agriculture, where soil diversity and the abundance of AMF are often reduced (Verbruggen *et al.* 2010; de Vries *et al.* 2013). Hence, our results indicate that agricultural sustainability, and in particular nitrogen use efficiency, could be enhanced by management practices that support soil life such as reduced tillage, crop rotation or mulching (Wardle 1995; Giller *et al.* 1997).

In the ENR treatment, the soil P pools were significantly reduced and the amount of additional P mobilized from

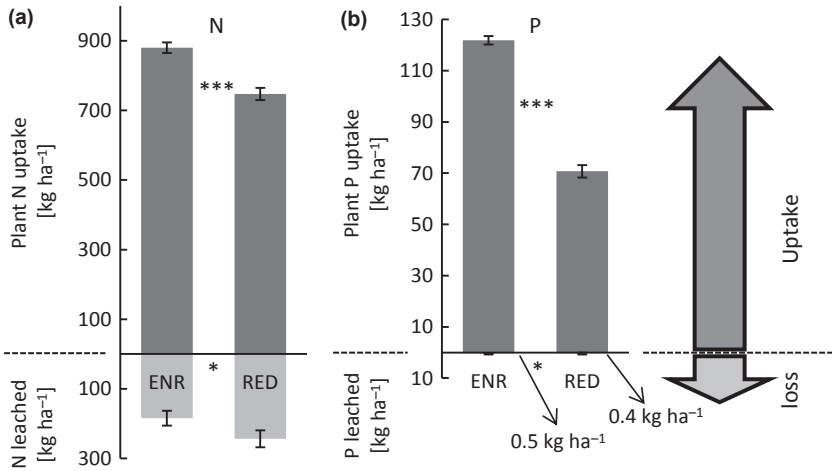

Fig. 5. Cumulative plant N uptake and N leaching (a) and plant P uptake and leaching (b) of plants grown in lysimeters inoculated with an enriched (ENR) or reduced (RED) soil-life inoculum during the whole experimental period. Error bars show ± 1 standard error (n = 8). Significant differences between the ENR treatment or RED treatment are indicated by asterisks (***, $P < 0{\cdot}001$; *, $P < 0{\cdot}05$).

Table 4. AMF colonization measures of the different crops, microbial biomass C and N contents and the respective statistical test results for lysimeters inoculated with an enriched (ENR) or reduced (RED) soil-life inoculum. Means are shown ± 1 standard error (n = 8)

	ENR	RED	d.f.	χ^2	P-value
AMF root colonization (%)*					
Maize/grass					
Total	70·8 (2·55)	10·25 (4·73)	1	11·3	0·001
Vesicles	1·63 (0·60)	2·88 (1·47)	1	0·00	0·957
Arbuscles	26 (2·33)	14·38 (3·16)	1	5·85	0·016
Wheat					
Total	88·63 (1·91)	28·13 (9·39)	1	11·33	0·001
Vesicles	15·63 (1·65)	5·13 (2·89)	1	6·47	0·011
Arbuscles	15·8 (2·72)	3·63 (1·57)	1	9·70	0·002
Grass–clover					
Total	75·0 (2·75)	43·0 (8·95)	1	5·84	0·016
Vesicles	6·5 (2·15)	0·63 (0·50)	1	9·14	0·002
Arbuscles	26·13 (3·91)	0·75 (0·41)	1	11·65	0·001
	ENR	RED	d.f.	F-value	P-value
Microbial biomass†					
Year 1					
MibiC (t ha^{-1})	1·75 (0·06)	1·62 (0·08)	1,13	2·04	0·177
MibiN (t ha^{-1})	0·29 (0·01)	0·26 (0·02)	1,13	1·03	0·328
Year 2					
MibiC (t ha^{-1})	1·40 (0·04)	1·44 (0·05)	1,13	0·68	0·425
MibiN (t ha^{-1})	0·24 (0·01)	0·24 (0·01)	1,13	0·55	0·472

*Nonparametric Kruskal–Wallis tests were performed.
†ANOVA was performed.

non-AAE-extractable soil pools increased more than two-fold (Table 5). The observed increase in total phosphorus leaching is, thus, likely to be a by-product of enhanced mineralization and mobilization of soil P resources by soil biota. However, the amounts of P lost during a period of almost 2 years were small, and the difference, even if significant, between the ENR treatment and the RED treatment was only 116 g ha^{-1}. When considered in relation to the increase in total plant P uptake of more than 51 kg ha^{-1}, this amount of P appears negligible. The relative P losses compared to the amount of P taken up by the plant biomass were significantly lower in the ENR treatment than in the RED treatment, indicating that the P-use efficiency was increased in the presence of an enriched soil

Table 5. Soil parameters in the topsoil measured at the end of the experiment and the respective ANOVA results for lysimeters inoculated with an enriched (ENR) or reduced (RED) soil-life inoculum. Means are shown ± 1 standard error (n = 8)

Soil parameters	ENR	RED	d.f.	*F*-value	*P*-value
Humus (t ha^{-1})	162·76 (3·81)	156·22 (3·87)	1,13	1·44	0·252
Organic C (t ha^{-1})	94·24 (1·97)	90·46 (2·16)	1,13	1·70	0·215
Total N (t ha^{-1})	11·12 (0·26)	10·90 (0·29)	1,13	0·38	0·549
Available P (kg ha^{-1})	1·78 (0·06)	2·58 (0·09)	1,13	59·54	0·000
P_{AAE} (kg ha^{-1})	68·59 (2·28)	87·59 (3·98)	1,13	19·64	0·001
K_{AAE} (kg ha^{-1})	606·6 (6·90)	606·9 (15·70)	1,13	0·00	0·989
Mg_{AAE} (t ha^{-1})	10·48 (0·13)	10·13 (0·15)	1,13	2·94	0·110
Ca_{AAE} (t ha^{-1})	117·03 (3·07)	118·1 (5·91)	1,13	0·03	0·866
pH (H_2O)	7·96 (0·02)	7·95 (0·02)	1,13	0·35	0·564
P_{mob} (kg ha^{-1})	62·78 (3·16)	30·54 (4·37)	1,13	38·95	0·000

P_{mob}, P mobilized from initially non-AAE-extractable soil P.

life. The dimensions of total plant N and P uptake and total N and P leaching are visualized in Fig. 5.

Enriched soil life significantly increased maize and wheat biomass and P concentrations, as well as maize N contents. These results imply that soil biota have the potential to improve the quantity and quality of agricultural yields. Plant N concentrations were, however, not affected. Plant N : P ratios have been proposed as a tool to assess nutrient limitation of plant communities (Koerselman & Meuleman 1996). The N : P ratios in maize and wheat plant tissue were all below 14, indicating N limitation, but they were significantly lower in the ENR treatment (Tables 2 and S2, Supporting information). This was mainly driven by a strong increase in plant P uptake in the ENR treatment. The multiple regression models indicate that AMF played an important role in increased plant P nutrition, while no direct effects of AMF root colonization on plant N uptake and biomass were observed. Hence, by significantly improving P nutrition, AMF probably increased plant N limitation and, thus, created an N drain towards plant biomass, providing an explanation for the reduction in N leaching.

It has also been shown that AMF can enhance N interception from soil, store substantial amounts of N in their extraradical hyphae and transport N to plants (Johansen, Jakobsen & Jensen 1992; Hodge & Fitter 2010), which could provide an additional mechanism for reduced N leaching. However, the results of this study do not reveal a direct effect of AMF on N cycling. The inoculation treatment effect also significantly explained a portion of plant P uptake, as well as of plant biomass in the multiple regression. This indicates that factors other than AMF root colonization contributed to the results.

On average, 107 kg N ha^{-1} was leached from soil per year. This is at the upper limit of estimated N leaching losses for many European countries (Leip *et al.* 2008). P leaching losses were low and comparable to other studies analysing P leaching losses in agricultural systems (Ulén 1999; Neumann, Torstensson & Aronsson 2012). The relatively high N leaching losses could be attributed to enhanced nutrient availability through soil sterilization (McNamara *et al.* 2003) and enhanced nutrient release from organic matter mineralization due to a higher soil temperature in the lysimeters (Kirschbaum 1995). In a Swiss lysimeter experiment using nonsterile soil, comparable amounts of leaching losses were reported (Spiess, Prasuhn & Stauffer 2011).

Maize yield was higher compared to yields commonly achieved in Swiss agriculture (Dubois *et al.* 1995; Rüegg *et al.* 1998). Enhanced nutrient availability in the lysimeters and 'edge effects' such as reduced competition from neighbouring plants for nutrients and light compared to field conditions probably contributed to the high yields.

The average root length colonized by AMF during the whole experiment amounted to 78% in the ENR treatment and 27% in the RED treatment. The root colonization levels in the RED treatment were comparable to Swiss fields under conventional management, while the root colonization in the ENR treatment was considerably higher than values found in Swiss organic fields (40–50% of root length colonized (Honegger *et al.* 2014)). However, AMF root colonization in the RED treatment reached values of 40% root length colonized in the second year, a value comparable to colonization levels in Swiss organic fields.

Windborne contamination from soil particles, microbes from neighbouring fields and rain splash are the most likely causes for the increase in AMF abundance in the RED treatment. Increased import of AMF and micro- and mesofauna to the RED treatment might also explain the less-pronounced differences between the treatments in the second year.

The ENR treatment consisted of soil biota with a size of ≤2 mm, while the RED treatment received soil microorganisms passing through an 11-μm filter, including some protozoa. The effects shown here must have been induced by soil organisms ranging between 11 μm and 2 mm in size, that is meso- and microfauna and AMF. Earlier studies showed that soil mesofauna, comprised of organisms like collembola and mites, can break down organic matter and release mineral nutrients into soil (Brussaard *et al.* 1995; Bardgett & Chan 1999). Members of soil

microfauna, including fungal, bacterial or root-feeding protozoa and nematodes, can increase nutrient mineralization, making nutrients available to plants (Woods *et al.* 1982; Griffiths 1986). AMF most likely played an important role in recycling nutrients released into the soil by enhancing nutrient interception and nutrient transfer to the plants. Furthermore, effects due to changes in plant physiology, or differences in the microbial communities arising from different ecological processes and different food webs developing within the inoculums and treatments, could have had an influence. With the approach employed in this study, it was not possible to precisely identify the organisms, interactions and processes responsible for the effects on plant yield and nutrient cycling. Future work should manipulate specific functional groups to elucidate specific mechanisms.

Several studies report that soil life can be enhanced by management practices that increase the organic matter content of soils and reduce soil disturbance. For example, collembolans, mites and nematodes were shown to be favoured by strip tilling combined with cover cropping (Wang, Hooks & Marahatta 2011), and the abundance or biomass of protozoa and nematodes is often found to be increased in organically managed soils compared to conventionally managed soils (Foissner 1992). Additionally, management practices like stubble retention and reduced tillage were shown to increase the abundance of protozoa, nematodes, collembolan and mites (Roper & Gupta 1995). Organic farming in general is often reported to have positive effects on soil biota (Mäder *et al.* 2002; Bengtsson, Ahnström & Weibull 2005). Reduced tillage and reduced fertiliser inputs, especially for P, have been shown to promote the abundance of AMF (Helgason *et al.* 1998; Kahiluoto, Ketoja & Vestberg 2000) and the ability of AMF to support plant P uptake (Köhl, Oehl & van der Heijden 2014). In the most intensively managed fields in the Netherlands, AMF were absent or nearly absent (Verbruggen *et al.* 2010). Such conditions might be comparable to our RED treatment in the first year of the experiment where AMF abundance was low and where we observed very high nutrient losses. In this respect, our results suggest that by applying managing practices that promote soil life, the nutrient-use efficiency in cropping systems can be enhanced, resulting in reduced environmental impacts and increased sustainability.

In a large-scale correlative field study by de Vries *et al.* (2013), it was reported that AMF may contribute to reduced N leaching in agricultural land-use systems. In a different study, it was shown that AMF can act as regulators of emissions of the strong greenhouse gas N_2O from soil (Bender *et al.* 2014), while another study (Wagg *et al.* 2014) showed that a decline in soil biodiversity can negatively affect several ecosystem functions. These results are in line with the results presented here and indicate that high nutrient losses in intensively managed fields may partly result from the disruption of soil food webs. Further work should now specifically test whether agricultural sustainability and nutrient-use efficiency are higher in agricultural fields with enriched soil life.

Phosphorus fertilizers are often applied in excess, because a large fraction of the applied P quickly reacts with the soil environment rendering it unavailable to plants (Barberis *et al.* 1995). Our results demonstrate that in the presence of soil biota, soil P resources normally unavailable to plants can efficiently be mobilized. Therefore, P fertilization could be reduced, sparing globally limited P resources.

The data presented here point to the fundamental role soil biota play in nutrient cycling. It is known that specific agricultural management practices (e.g. reduced tillage, reduced fertilization, crop rotation and organic farming) can promote soil life and biodiversity in general (Tuck *et al.* 2014). While such management practices often show reduced yields compared to conventional, more intensive practices (Gabriel *et al.* 2013), the results obtained in this study suggest that that there exist benefits beyond enhanced diversity. Increased nutrient-use efficiency obtained through positive effects on soil biota potentially reduces fertilizer amounts needed for agricultural production and reduces nutrient losses to the environment through leaching, representing a major thread to the earth system (Rockström *et al.* 2009). There is an urgent need to conduct field-based studies comparing agricultural fields with high and low soil biota abundance and diversity to test whether nutrient-use efficiency is higher when soil communities are well developed.

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Data accessibility

All data presented in this manuscript are available at Dryad Digital Repository: http://doi.org/10.5061/dryad.p5v4g (Bender & van Der Heijden 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Supporting methods.

Fig. S1. Maize plants growing in the lysimeters.

Table S1. Nutrient solution used for inoculum production.

Table S2. Leaching losses, plant biomass, nutrient contents and the respective ANOVA results for lysimeters inoculated with an enriched (ENR) or reduced (RED) soil-life inoculum in year 1.

Table S3. Cumulative leaching losses, plant biomass and nutrient contents for the whole experimental period.

Table S4. MANOVA output investigating effects of Block and Inoculation treatment on nutrient leaching and plant performance.

Table S5. Results of multiple regressions explaining total plant P content, total plant N content and total plant biomass for the whole experiment.