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Research article

Yield and arbuscular mycorrhiza of winter rye in a 40-year fertilisation trial

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Abstract – The impact of different fertilisation treatments on soil organic matter, available soil nutrients, mycorrhizal and root properties, as well as on the yield response of winter rye (*Secale cereale*) was studied in a long-term field trial in Austria under dry site conditions. Winter rye has been grown since 1906 in soils treated with easily soluble mineral fertiliser, farmyard manure, and in an unfertilised control. We found the soil organic matter to be 96% higher in the plots fertilised with farmyard manure compared with easily soluble mineral fertiliser. Available soil phosphorous and potassium contents were at least 136% higher in both fertilised treatments than in the unfertilised control. Arbuscular mycorrhizal colonisation (+46%) of winter rye roots by indigenous arbuscular mycorrhizal fungi, arbuscule frequency (+20%), and the length of the extraradical arbuscular mycorrhizal mycelium (+18%) were higher in the unfertilised control and reduced in the NPK treatment compared with the farmyard manure treatment. The average grain yield of winter rye from 1960 to 2000 increased in all treatments. This increase was higher in the fertilised treatments, +41% for farmyard manure and +60% for easily soluble mineral fertiliser, than in the unfertilised control. Two main effects presumably accounted for the continuously increasing average winter rye yield in all fertilisation treatments: (1) the use of modern winter rye varieties with a higher nutrient efficiency; and (2) an ongoing atmospheric nitrogen deposition. We conclude that the preferential application of farmyard manure, typical for low-input farming systems, resulted in increased levels of soil organic matter, arbuscular mycorrhizal colonisation and arbuscule frequency, supporting soil fertility by an enhanced crop nutrient uptake by arbuscular mycorrhizal fungi under dry site conditions, thus promoting crop yield stability and sustainable plant growth.

arbuscular mycorrhizal fungi (AMF) / long-term field trial / continuous rye cropping / farmyard manure (FYM) / easily soluble mineral fertiliser (NPK)

1. INTRODUCTION

Recent adverse environmental effects of high-input agricultural systems, e.g. pollution of groundwater due to the application of easily soluble mineral fertilisers, have increased the public interest in sustainable agricultural systems such as organic farming, where soluble mineral fertilisers are prohibited (Oberson and Frossard, 2005). Symbioses of crop plants with soil micro-organisms are crucial for soil fertility and crop nutrition in low-input farming systems. The effects of low-input management practices such as organic manuring on soil microbial communities need to be understood to increase soil fertility for sustainable crop production (Mäder et al., 2002).

Applying farm yard manure (FYM) has a long-term effect on physical soil properties and soil micro-organisms (Mäder et al., 1995). It usually takes several decades until a dynamic equilibrium in the content and quality of soil organic matter has been reached (Reganold et al., 1987). Therefore, long-term field trials are required to assess the sustainability of organic matter-based management practices on crop yield and soil fertility. Only a few results, however, are available about soil nutrient contents, arbuscular mycorrhizae and crop yield from long-term fertilisation field trials.

In dry soils, the mobility of nutrients in soil solution and the microbial activity that mobilises mineral nutrients from the solid phase are generally reduced. Under such dry conditions, the nutrient availability to crops may be insufficient, especially in low-input agricultural systems.

An arbuscular mycorrhiza is a symbiotic relationship between arbuscular mycorrhizal fungi and plant roots (Smith and Read, 1997). Most crop plants in temperate agricultural systems build an arbuscular mycorrhiza; therefore, arbuscular

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mycorrhizal fungi play a key role in nutrition, water relations, and in resistance against plant pathogens and diseases of crop plants (Jeffries et al., 2003), particularly in organic farming (Gosling et al., 2006). Furthermore, arbuscular mycorrhizal fungi can improve plant growth, especially in dry soils (Augé, 2001). Agronomic practices such as fertiliser application, tillage or crop rotation have major effects on arbuscular mycorrhizal fungi communities (Galvez et al., 2001; Oehl et al., 2003). Applying organic fertilisers such as farmyard manure can enhance the arbuscular mycorrhizal diversity and colonisation of host plants compared with inorganic fertilisers (Allen et al., 2001; Oehl et al., 2004). Little is known, however, about long-term effects of different fertilisation treatments on arbuscular mycorrhizal colonisation of crops under dry site conditions.

Our study therefore evaluates the effects of easily soluble mineral fertilisers, farmyard manure and an unfertilised control on soil nutrient content and on the relation between arbuscular mycorrhizal colonisation with indigenous arbuscular mycorrhizal fungi and the yield of winter rye (*Secale cereale*). The approach involves a long-term field trial under dry site conditions.

2. MATERIALS AND METHODS

2.1. Study site

The study was performed in a long-term field trial conducted by the Department of Applied Plant Sciences and Plant Biotechnology on the experimental Farm of the University of Natural Resources and Applied Life Sciences of Vienna in Gross-Enzersdorf, Austria, in the year 2000. The long-term field trial is located north-east of Vienna (48°11'N, 16°33'E) in the so-called "Marchfeld", an area with about 100 000 hectares of farmland at an altitude of 150–160 m above sea level. The soil on the study site is a Calcaric Phaeozem from Loess and alluvial fine sediment with a silty loam texture (25% clay, 64% silt, 11% sand) and a pH ($CaCl_2$) of 7.6. The climate in the Marchfeld is pannonic, characterised by hot, dry summers with little dew, and cold winters with little snow. The long-term average (1971–2000) annual precipitation is 520 mm; the mean annual temperature is 9.8 °C. In the cropping season 1999/2000, the total precipitation amounted to only 501 mm, whereas the annual mean temperature (11.2 °C) was well above the average.

2.2. Experimental design and management

The long-term field trial was started in 1906 to test the possibility of completely replacing farmyard manure by easily soluble mineral fertilisers, and whether different fertilisation systems can compensate for crop rotation deficits from continuous cropping of winter rye (Steineck and Ruckenbauer, 1976).

The field trial comprised an area of 4000 m^2, which is divided into four long plots of 1000 m^2, each with three equal sub-plots (20 m × 13 m), and split into two parts (Fig. 1).

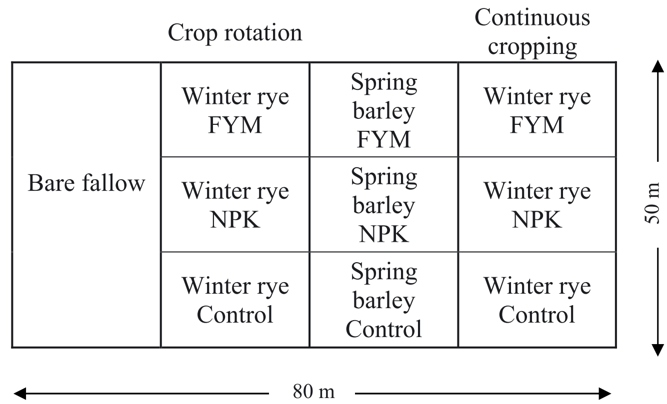

Figure 1. Design of the long-term field trial in Gross-Enzersdorf for the continuous cropping of rye in 2000. FYM: farmyard manure; NPK: easily soluble mineral fertiliser (nitrogen, phosphorus, potassium); Control: no fertiliser applied; White sub-plots: bare fallow, winter rye and spring barley in crop rotation; Shaded sub-plots: continuous cropping of winter rye.

Table I. Applied nutrients in the long-term field trial in Gross-Enzersdorf. NPK: easily soluble mineral fertiliser; FYM: farmyard manure, FYM is applied as 20 000 kg ha^{-1} fresh weight.

Treatment	N	P	K
	($kg\ ha^{-1}\ a^{-1}$)		
Control	0	0	0
NPK	117	44	125
FYM	117	44	125

In the first three neighbouring plots, an old crop rotation (bare fallow – winter rye – spring barley) has been performed since 1906, whereas continuous rye cropping has been carried out on the fourth plot (grey shaded). Each plot was divided into 3 sub-plots, one not fertilised at all (control). The second plot was exclusively treated with easily soluble mineral fertiliser (NPK), i.e. Nitramoncal (27% water-soluble nitrogen as nitrate and ammonium), superphosphate (16% water-soluble phosphorous as P_2O_5) and potassium chloride granulate (60% water-soluble K_2O). The remaining, third plot was treated only with farmyard manure (FYM). The plots with bare fallow were not fertilised. The average annual fertilisation rate was the same in the farmyard manure and easily soluble mineral fertiliser treatments (Tab. I).

The plots in the cropping season of the experiment were sown with the Austrian rye variety "Tschermaks veredelter Marchfelder" at a rate of 500 kernels m^{-2}, equivalent to 175 kg ha^{-1}. The straw was completely removed from the plots after the harvest every year.

2.3. Soil and root sampling and analysis

Four sub-samples consisting of 5 soil cores at 2 soil depths (3 cm diameter, sampling depth 0–10 and 10–30 cm) were taken from each sub-plot at shooting of the rye plants in early

May 2000. This date was chosen because cereals show the highest uptake rates of phosphorous until shooting (Römer and Schilling, 1986). Soil mineral nitrogen content was assessed as the total of ammonium and nitrate content. The soil properties were determined according to Austrian Standards. The rye roots were separated from the adhering soil by a hydro-pneumatic elutriation system (Gillison's Variety Fabrication Inc., USA) through a 560-μm mesh sieve. The root density ($cm_{root}\ cm^{-3}_{soil}$) was determined according to Giovanetti and Mosse (1980). Roots were stained according to Vierheilig et al. (1998), and the arbuscular mycorrhizal colonisation (%) was determined under a light microscope according to McGonigle et al. (1990). The extraradical mycelium length was estimated using a modified membrane filtration technique (Jakobsen et al., 1992). In addition, the frequency of arbuscules (%) was observed, because in this fungal structure nutrient exchange between the host plant and arbuscular mycorrhizal fungi occurs. Considering that plants can compensate for a low arbuscular mycorrhizal colonisation by higher root density (Newsham et al., 1995), the root density was multiplied by the mycorrhizal colonisation to obtain the mycorrhizal density ($cm_{colonised\,roots}\ cm^{-3}_{soil}$). This is a more reliable parameter for the mycorrhizal colonisation of a plant root system (Amijee et al., 1989). Arbuscular mycorrhizal fungi spores were isolated by a modified water/sucrose centrifugation method (Ianson and Allen, 1986).

2.4. Plant sampling and analysis

The crop yield, calculated as 86% dry matter, was determined by manually cutting one square metre in three replicates per plot in July 2000. The average grain yield was determined by an experimental combine harvester.

2.5. Statistical analysis

The long-term field trial was set up in only one replicate per variant in 1906. To test for fertilisation effects, the two cropping system treatments (continuous rye, crop rotation) were regarded as replicates. The data obtained for the soil properties and the mycorrhizal properties were subjected to a two-way analysis of variance (ANOVA) for the fertilisation system and soil depth. The yield properties in 2000 as well as the average grain yield from 1960 to 2000 were subjected to a one-way analysis of variance (ANOVA) for the fertilisation system. Mean values of all properties were compared using the Student-Newman-Keuls Procedure ($P < 0.05$). The homogeneity of variance and the normal distribution of the residues were tested. If necessary, the original values were log-transformed to meet the requirements of the ANOVA. Regression analysis was used to test differences in grain yield increase between fertiliser treatments during the experimental period. All statistical analyses were carried out with SPSS 15.0.

Table II. Soil chemical properties of winter rye fertilisation treatments. N = 12; NPK: easily soluble mineral fertiliser; FYM: farmyard manure; SD: standard deviation; mean values within a line with the same letter do not differ significantly (Student-Newman-Keuls Procedure, $P < 0.05$).

Property	Control		NPK		FYM	
Soil organic matter (%)	3.3	b	2.4	b	4.7	a
SD ±	0.6		0.7			
Magnesium (mg kg^{-1})	123	b	118	b	210	a
SD ±	5		10		8	
Phosphorous (mg kg^{-1})	44	b	104	a	117	a
SD ±	4		18		9	
Potassium (mg kg^{-1})	364	b	864	a	973	a
SD ±	30		150		75	

3. RESULTS AND DISCUSSION

3.1. Soil chemical properties

The long-term application of different fertilisers affected the soil chemical properties in several ways. Fertiliser treatments did not affect the mineral nitrogen content (as a total of ammonium and nitrate nitrogen) or the soil carbonate content of samples taken in May 2000 (data not shown). Fertilisation with farmyard manure increased soil organic matter contents (+96% and +42%, respectively) and magnesium contents (+78% and +70%, respectively) compared with easily soluble mineral fertiliser and the unfertilised control treatment (Tab. II). Soil phosphorous and potassium contents, however, were higher in both fertilised treatments (easily soluble mineral fertiliser: +136% and +137%, respectively; farmyard manure: +166% and +167%, respectively) than in the unfertilised control (Tab. II).

3.2. Mycorrhizal properties

Arbuscular mycorrhizal colonisation (–32% and –58%, respectively) and arbuscule frequency (–17% and –26%, respectively) in winter rye roots, as well as the length of the extraradical mycelium (–16% and –38%, respectively) in the soil, were reduced in the farmyard manure and easily soluble mineral fertiliser treatments versus the unfertilised control (Tab. III), which had the lowest soil phosphorous contents (Tab. II).

Arbuscular mycorrhizal colonisation is known to increase at reduced phosphorous availability (Ryan and Ash, 1999; Ryan and Graham, 2002) and in organic fertilisation treatments, mainly in poor soils (Muthukumar and Udaiyan, 2000).

In accordance with Joner (2000) and Mäder et al. (2000), we found a higher arbuscular mycorrhizal colonisation of winter rye roots in the plots fertilised with farmyard manure versus those fertilised with NPK (Tab. III). Soil phosphorous contents cannot explain this difference because they did not differ significantly between the two treatments (Tab. II).

Three synergistic effects of organic matter addition can explain the higher arbuscular mycorrhizal colonisation level and

Table III. Mycorrhizal properties of winter rye fertilisation treatments. N = 12; NPK: easily soluble mineral fertiliser; FYM: farmyard manure; AM: arbuscular mycorrhiza; SD: standard deviation; mean values within a line with the same letter do not differ significantly (Student-Newman-Keuls Procedure, $P < 0.05$).

Property	Control		NPK		FYM	
AM colonisation (%)	38	a	16	c	26	b
SD ±	2		3		2	
Arbuscule frequency (%)	84	a	62	c	70	b
SD ±	1		2		2	
Extraradical mycelium (mm g^{-1})	2573	a	1591	c	2174	b
SD ±	137		78		65	

Table IV. Two-way ANOVA (fertilisation, soil depth) levels of significance of soil and mycorrhizal properties of winter rye fertilisation treatments. N = 12; values <0.05 are significant.

Property	Fertilisation	Soil depth	Fertilisation*Soil depth
Soil organic matter	0.007	0.504	0.947
Magnesium	0.000	1.000	0.385
Phosphorous	0.000	0.195	0.819
Potassium	0.000	0.195	0.819
Mycorrhizal colonisation	0.000	0.048	0.724
Arbuscule frequency	0.000	0.134	0.729
Extraradical mycelium	0.000	0.085	0.610
Mycorrhizal density	0.125	0.010	0.460

arbuscule frequency in the long-term farmyard manure fertilisation treatments compared with the NPK fertilisation: (1) higher soil organic matter contents apparently support the survival of arbuscular mycorrhizal fungi propagules during the bare fallow period before winter rye in the crop rotation; (2) especially in nutrient-poor soils, fertilisation with farmyard manure can support the arbuscular mycorrhizal colonisation of crops by avoiding a deficiency of carbohydrates from the photosynthesis of the crop plants for the AMF; (3) farmyard manure enhances soil organic matter, improving soil biological, physical and chemical properties (McCoy 1998). This supports populations of beneficial soil micro-organisms, maintains soil structure, and improves water retention capacity (Arden-Clarke and Hodges, 1988).

In a previous analysis of our experiment, Steineck and Ruckenbauer (1976) calculated that the continuous supply of 20 000 kg farmyard manure ha^{-1} a^{-1} enriched the soil with about 2500 kg C ha^{-1} a^{-1}. The fertilisation with farmyard manure for 94 years thereby improved the conditions for plant root growth and may have also positively affected mycorrhizal colonisation (Tu et al., 2006). Adding NPK, on the contrary, does not directly add organic matter to the soil. Indirectly, fertilisers increase crop yield and thereby also crop residues. This effect, however, is much smaller compared with the amount of organic matter supplied with FYM.

The arbuscular mycorrhizal fungi from unfertilised soils were shown to promote their host plant nutrient uptake because of a higher number of arbuscules (the site of nutrient transfer to the host) compared with arbuscular mycorrhizal fungi from soils fertilised with soluble inorganic fertilisers for several years (Johnson, 1993).

The root density, mycorrhizal density and arbuscular mycorrhizal fungi spore density did not differ significantly between the treatments (data not shown). Spore populations are at their lowest during the growing season. Therefore, the number of spores per soil volume is not an expressive parameter for arbuscular mycorrhizal fungi populations when plants flower.

Mycorrhizal colonisation and mycorrhizal density were higher in the lower soil layer (10–30 cm), but no significant interactions between fertilisation treatments and soil depth occurred (Tab. IV).

3.3. Yield properties

The long-term field trial was set up in 1906. Data on the experiment before the 1950s, however, are rare or missing.

The fertile tillers in the farmyard manure treatment (661 m^{-2}) as well as in the easily soluble mineral fertiliser treatment (589 m^{-2}) were higher than in the unfertilised control (445 m^{-2}). Also, the grain yields in 2000 were higher in the two fertilised treatments (4242 and 4403 kg ha^{-1}, respectively) than in the unfertilised control (2320 kg ha^{-1}). Nitrogen from easily soluble mineral fertiliser is generally better available to plants than nitrogen from FYM. This is probably the main reason for higher grain yields of rye in the easily soluble mineral fertiliser treatment.

The mean grain yield from 1960 to 2000 was higher in the plots fertilised with easily soluble mineral fertiliser (3630 kg ha^{-1}) and lower in the unfertilised control (2266 kg ha^{-1}) compared with the farmyard manure treatment (3197 kg ha^{-1}). The yield in 2000 was above the long-term means from 1960 to 2000 (Tab. V).

Table V. Yield properties of winter rye fertilisation treatments. NPK: easily soluble mineral fertiliser; FYM: farmyard manure. For fertile tillers, straw and grain yield in the year 2000: N = 6. For average grain yield from 1960 to 2000: N = 246; mean values within a line with the same letter do not differ significantly (Student-Newman-Keuls Procedure, $P < 0.05$); SD: standard deviation.

Property	Control		NPK		FYM	
Fertile tillers in the year 2000 (m^{-2})	445	b	589	a	661	a
SD ±	38		39		95	
Straw yield in the year 2000 ($g\ m^{-2}$)	535	b	852	a	618	b
SD ±	168		48		230	
Grain yield in the year 2000 ($kg\ ha^{-1}$)	2320	b	4403	a	4242	a
SD ±	857		433		497	
Average grain yield from 1960 to 2000($kg\ ha^{-1}$)	2266	c	3630	a	3197	b
SD ±	636		935		844	

Table VI. Pearson correlation coefficients of winter rye yield and differences in the yield of winter rye fertilisation treatments from 1960 to 2000. N = 81; * $P < 0.05$, ** $P < 0.01$. NPK: easily soluble mineral fertiliser; FYM: farmyard manure; Control: unfertilised treatment.

Winter rye yield			Differences in the yield of winter rye		
Control	NPK	FYM	NPK-Control	FYM-Control	NPK-FYM
0.289**	0.605**	0.531**	0.378**	0.294**	0.164

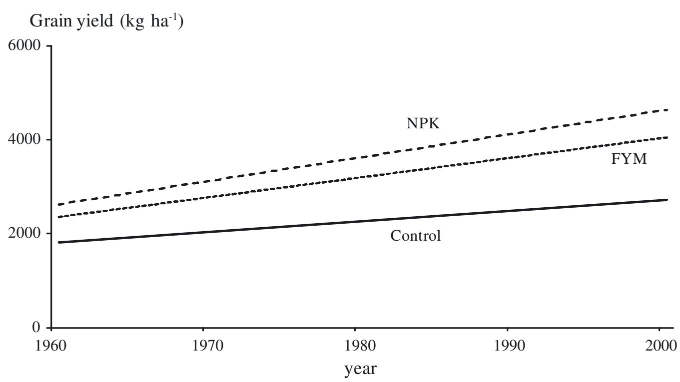

Figure 2. Grain yield of fertilised and unfertilised winter rye from 1960 to 2000. NPK: easily soluble mineral fertiliser; FYM: farmyard manure; Control: unfertilised treatment.

The total precipitation during the cropping season of our experiment (501 mm) was below the long-term average (520 mm). Arbuscular mycorrhizal fungi are known to increase the drought resistance of their host plant (Augé, 2001). This effect should reduce drought-induced yield depressions in the unfertilised control and farmyard manure treatments with increased mycorrhizal colonisation rates compared with the easily soluble mineral fertiliser treatment. The trend of reduced standard deviation values for average grain yields from 1960 to 2000 in these two treatments, compared with the easily soluble mineral fertiliser treatment (Tab. V), is in line with this expected effect. It did not occur, however, in the year 2000.

The average yield of rye increased from 1960 to 2000 in all fertilisation treatments, even in the unfertilised rye plots (Fig. 2). This increase was more pronounced in the fertilised treatments than in the unfertilised control (Tab. VI).

This is presumably due to two main effects: (1) the use of modern ("Motto", "Elect", "Danko" and "Tschermaks veredelter Marchfelder") instead of old ("Tschermaks Marchfelder") winter rye varieties with a higher nutrient efficiency; (2) an on-going wet and dry atmospheric deposition of nitrogen, to mean values of about 13 kg N ha^{-1} a^{-1} (as a total of ammonia and nitrate) during the last decades, indicated by measurements of Puxbaum and Gregori (1998).

4. CONCLUSION

Different long-term fertilisation treatments, i.e. easily soluble mineral fertiliser, farmyard manure and an unfertilised control, clearly affected soil nutrient contents, mycorrhizal properties and the yield of winter rye in our study.

We showed that, compared with easily soluble mineral fertiliser, the preferential application of farmyard manure – typical for low-input farming systems – resulted in increased levels of soil organic matter, arbuscular mycorrhizal colonisation and arbuscule frequency, as a basis for a stimulating arbuscular mycorrhizal effect on nutrient uptake. This is particularly important when nutrient availability is low, i.e. under conditions of low-input agricultural systems that renounce or minimise soluble mineral *P* fertilisers, as well as in dry soils. Such long-term application of farmyard manure supports soil fertility by enhancing crop nutrient uptake by arbuscular mycorrhizal fungi, especially under dry site conditions. This, in turn, supports crop yield stability and sustainable plant growth.

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