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Effect of management and age of ploughed out grass–clover on forage maize yield and residual soil nitrogen

M. Cougnon[1], K. Van Den Berge[2,3], T. D'Hose[4], L. Clement[2,3] and D. Reheul[1]

[1]Department of Plant and Crops, Faculty of Bioscience Engineering, Ghent University, Proefhoevestraat 22, 9090 Melle, Belgium; [2]Department of Applied Mathematics, Computer Science and Statistics, Ghent University, Krijgslaan 281 (S9), Belgium; [3]Bioinformatics Institute Ghent, Ghent University, 9000 Ghent, Belgium and [4]Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit, Crop Husbandry and Environment, Burg. Van Gansberghelaan 109, 9820 Merelbeke, Belgium

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Author for correspondence:
M. Cougnon,
E-mail: mathias.cougnon@ugent.be

Abstract

Forage maize (*Zea mays* L.) is often grown year after year on the same land on many intensive dairy farms in north-west Europe. This results in agronomical problems such as weed resistance and decline of soil quality, which may be solved by ley-arable farming. In the current study, forage maize was grown at different nitrogen (N) fertilization levels for 3 years on permanent arable land and on temporary arable land after ploughing out different types of grass–clover swards. Swards differed in management (grazing or cutting) and age (temporary or permanent). Maize yield and soil residual mineral N content were measured after the maize harvest. There was no effect on maize yield of the management of ploughed-out grass–clover swards but a clear effect of the age of grass–clover swards. The N fertilizer replacement value (NFRV) of all ploughed grass–clover swards was >170 kg N/ha in the first year after ploughing. In the third year after ploughing, NFRV of the permanent sward still exceeded 200 kg N/ha, whereas that of the temporary swards decreased to 30 kg N/ha on average. Soil residual nitrate (NO_3^-) remained below the local, legal threshold of 90 kg NO_3^- N/ha except for the ploughed-out permanent sward in the third year after ploughing (166 kg NO_3^- N/ha). The current study highlights the potential of forage maize – ley rotations in saving fertilizer N. This is beneficial both for the environment and for the profitability of dairy production in north-western Europe.

Introduction

Perennial forage grass species and forage maize (*Zea mays* L.) are the main crops on many intensive dairy farms in north-west Europe. Maize is often grown in monoculture: year after year on the same land. Nevens and Reheul (2001) showed that maize yield remains fairly constant in monoculture under high nitrogen (N) input (180 kg N/ha/year). The negative effects of monoculture become more pronounced with lower chemical inputs: (i) decreasing yields (Nevens and Reheul, 2001; Franzluebbers *et al.*, 2014) and lower yield stability (Borrelli *et al.*, 2014), (ii) problems with weed suppression, in particular panicoid weed grasses such as *Panicum* spp., *Echinochloa* spp., *Setaria* spp. and *Digitaria* spp. (De Cauwer *et al.*, 2012; Claerhout *et al.*, 2015) and (iii) a decrease of soil fertility from a decline in soil organic matter (SOM) and soil biological quality (Sleutel *et al.*, 2003; Goidts and Van Wesemael, 2007; Van Eekeren *et al.*, 2008; Piutti *et al.*, 2015), making these soils, e.g. more vulnerable to erosion (Franzluebbers *et al.*, 2014). Continuing legal restrictions regarding N input (maximum N input has been limited to 150 kg N/ha in Flanders in the period 2015–2018 (Flemish Land Agency, 2015)) and herbicide application not only have put an end to irresponsible high inputs but also have renewed interest in crop rotation.

Ley-arable farming is one type of crop rotation that may reduce reliance on chemical inputs (Vertés *et al.*, 2007; Eriksen *et al.*, 2010; Piutti *et al.*, 2015). Indeed, mineralization of the N present in ploughed-out stubble and grassroots may lead to high maize yields and high N yields with minimal N fertilization. This was demonstrated in a long-term experiment comparing ley-arable farming with crops on permanent arable land on a sandy loam soil in Belgium by Nevens and Reheul (2002). In addition, Borrelli *et al.* (2014) showed that growing grain maize in rotation with Italian ryegrass (*Lolium multiflorum* L.) increased yield stability compared with continuous maize cropping in a 26-year crop rotation experiment in the North of Italy. Ley-arable rotations may slow down the spread of herbicide-resistant weeds in maize, such as barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.), which is promoted by maize monoculture (Claerhout *et al.*, 2015).

The effect of ploughed-out grassland on the yield of following crops depends on many factors such as management, age, clover content, etc., of the grassland. The literature quantifies

Table 1. Average temperature (°C) measured in the meteorological station of Melle, Belgium during the arable period of the experiment

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
2008	6.7	5.3	6.6	9.0	15.8	16.0	17.8	17.6	13.4	10.3	7.0	2.8	10.7
2009	0.4	3.4	6.3	11.5	14.0	15.9	18.3	18.5	15.6	11.0	9.9	2.7	10.6
2010	0.0	2.5	6.8	9.7	11.0	16.8	19.8	17.1	14.0	10.7	6.3	−0.7	9.5
NORM	3.4	3.8	6.7	9.4	13.3	15.9	18.1	17.9	14.9	11.2	7.0	4.1	10.5

NORM: average temperature in period 1981–2010.

Table 2. Rainfall (mm) measured in the meteorological station of Melle, Belgium during the arable period of the experiment

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
2008	57.3	41.1	131.3	69.3	44.9	88.4	64.7	84.5	53.8	82.9	88.8	41.6	848.6
2009	56	56.9	70.4	31.6	75.3	65.9	77.7	23.1	27.3	87.1	142.2	100.3	816.8
2010	43.8	70.2	64.9	16.5	38.5	28.1	72.3	232.8	127.7	89.5	136.4	43.5	964.2
NORM	71.0	56.0	63.0	49.0	59.0	75.0	76.0	80.0	70.0	79.0	78.0	80.0	836.0

NORM: average rainfall in period 1981–2010.

these effects abundantly in ley-arable rotations (Webster *et al.*, 1999; Eriksen, 2001; Hansen *et al.*, 2005, 2007; Eriksen *et al.*, 2006, 2008; Christensen *et al.*, 2009). However, the majority of reported trials deal with (spring) cereals; fewer with maize (Nevens and Reheul, 2002; Kayser *et al.*, 2008; Borrelli *et al.*, 2014) and none compares the effect of both temporary and permanent grassland. An important issue when ploughing out grass swards is potential soil water pollution due to nitrate (NO_3^-) leaching (Eriksen *et al.*, 2008; Kayser *et al.*, 2008). Verloop *et al.* (2006), however, showed that by reducing fertilization levels in first- and the second-year maize crops on a sandy soil in the Netherlands by 90 and 45 kg N/ha, respectively, it was possible to grow grass–maize rotations without having higher NO_3^- leaching losses compared with maize monoculture.

The current trial comprises a ley-arable rotation with ploughed-out grass–clover of different ages followed by forage maize for three consecutive years. The grass–clover was either grazed or cut, which makes sense as the importance of grazing is decreasing markedly (Van den Pol-van Dasselaar *et al.*, 2015). The effect of N fertilization on maize yield was modelled for the different ploughed-out grass-clover swards. The following hypotheses were addressed:

(H1) Ploughed-out grazed grass–clover delivers more N to the forage maize than cut grass–clover. Indeed, lower net yields and ample N recycling through urine and dung might result in a higher amount of N in dead and living organic matter.

(H2) Ploughed-out permanent grass–clover delivers more N for a longer period to forage maize than temporary grass–clover.

Materials and methods

Site

The experiment was established on a sandy loam soil at the experimental farm of Ghent University at Melle, Gontrode, Belgium (50°59′ N, 03°49′ E, 11 m asl) in 2004. The levels of clay (<2 µm), silt (2–20 µm), fine sand (20–200 µm) and coarse sand (200–2000 µm) at the experimental location are 86, 116, 758 and 40 g/kg, respectively. Climate data can be found in Tables 1 and 2. Half of the field on which the experiment was established had a history of permanent grass–clover (PG) established for the first time in 1966. It was re-sown for the last time in 1999 with a mixture of perennial ryegrass (*Lolium perenne* L.; 40 kg/ha), red fescue (*Festuca rubra* L.; 24 kg/ha) and white clover (*Trifolium repens* L.; 5 kg/ha). The other half of the field had been permanent arable land (PA) for about 50 years and cropped continuously with forage maize since 1999. In September 2004, the arable half was reorganized: one-third continued to be arable, while two-thirds were sown with a mixture of perennial ryegrass (40 kg/ha) and white clover (5 kg/ha) and turned into temporary grassland (TG). The whole experimental field was divided into three blocks (60 × 18 m), each consisting of four plots (15 × 18 m each). The plots represented grazed permanent grassland (PGg) on the former permanent grassland part of the field; arable land (PA) and both grazed (TGg) and cut (TGc) temporary grassland on the former arable part of the field (Fig. 1).

Ley phase (2005–2007)

Cut temporary grassland was cut four times a year to a height of *c.* 0.05 m. The first cut occurred near the end of April, the following cuts at 6-week intervals. Annual fertilization per hectare for TGc was 300 kg N from calcium ammonium nitrate (270 g N/kg), 52 kg phosphorous (P) from triple superphosphate (206 g P/kg) and 246 kg potassium (K) from potassium chloride (KCl; 328 g K/kg), which corresponded to the legal N-limit for the experimental area. The fertilization was applied in fractions corresponding to the demand of grass–clover swards throughout the growing season: 80 kg N, 52 kg P and 123 kg K before the first cut, 70 kg N after the first cut, 70 kg N and 123 kg K after the second cut and 40 kg N after the third and fourth cuts.

The grazed grassland was rotationally stocked with dairy heifers in 2005–2007. The number of grazing animals was adapted to herbage mass such that the pastures were grazed for 4–5 days. A plot was grazed until the grass–clover sward height

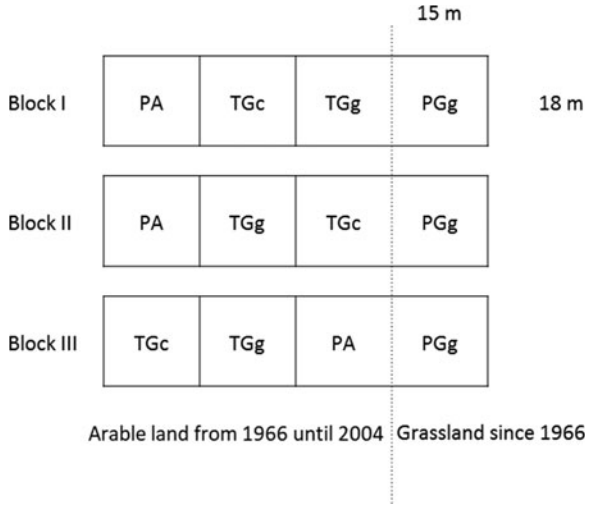

Fig. 1. Design of the experiment; situation in period 2005–2007. PA, permanent arable land; TGg, grazed temporary grass–clover; TGc, cut temporary grass–clover; PGg, grazed permanent grass–clover.

Table 3. Main soil chemical parameters measured in April 2008, at the beginning of the arable phase and recommended rage by the soil service of Belgium

	pH (KCl)	SOC	P (g/kg dry soil)	K
Permanent arable	5.7	10.0	0.247	0.179
Cut temporary grass–clover	5.3	9.9	0.223	0.229
Grazed temporary grass–clover	5.4	10.1	0.221	0.229
Grazed permanent grass–clover	5.0	17.0	0.186	0.149
Recommended	6.5–7.0	12–16	0.13–0.21	0.15–0.23

decreased to approximately 0.07 m (visually estimated). The heifers did not receive any feed supplement. Equal N, P and K fertilization was applied to both grazed and cut grassland, although fertilization on the grazed plots was corrected for the excretion of (mineral) N, P and K by heifers using the excretion values proposed by the Flemish Land Agency (Flemish Land Agency, 2015). This resulted in an annual fertilization per hectare of 200 kg N from calcium ammonium nitrate (270 g N/kg), 43 kg P from triple superphosphate (206 g P/kg) and 164 kg K from KCl (328 g K/kg) divided into 50 kg N, 43 kg P and 82 kg K at the beginning of April, before grazing started, and 50 kg N after the first three grazing rounds. The remaining 82 kg K was applied after the second grazing round. No fertilization was applied after the fourth and last grazing round. Average annual share of white clover in the ground cover fluctuated between 0.1 and 0.2 in PG and between 0.2 and 0.3 in TG (estimated by visual scores).

The PA plot was grown with forage maize (cv. LG32.15) in 2005, 2006 and 2007. Annual fertilization was 200 kg N/ha, 34 kg P/ha and 205 kg K/ha. Plots were sown in the last 10 days of April at a density of 114 000 seeds/ha and harvested in September. Weeds were killed post-emergence using sulcotrione, dimethenamid-P and nicosulfuron.

No yields or quantitative data were recorded during the ley phase since this was outside the scope of the current study, but dry matter yields (DMY) of forage maize and grass–clover under these growing conditions can be estimated as 18 and 14 t/ha, respectively.

Arable phase (2008–2010)

In February 2008, all TG and PG plots were converted into temporary arable land (TA). The swards were killed with glyphosate (1440 g/ha) and rotavated 2 weeks later. In April 2008, soil samples (0–0.3 m) were taken from each plot ($n = 10$) and single pooled samples for PA, TGg, TGc and PGg were analysed for pH in KCl, soil organic carbon (SOC), P and K (Table 3). To measure pH-KCl, a sub-sample of 20 ml oven-dried soil (70 °C, 16 h), was added to an open polyethylene cup (200 ml volume) and 100 ml of a 1 M KCl solution was added. The suspension was thoroughly mixed and shaken for 1 h on a rotary shaker (150 rpm). After an additional 2 h of settling, the pH-KCl was measured with a pH electrode (Consort, C832, Turnhout, Belgium), immediately after stirring the soil suspension (ISO 10390, 2005). The SOC content was measured in a TOC-analyser (Skalar, Primacs, Breda, The Netherlands), directly on an oven-dried (70 °C, 16 h) sub-sample of 1000 mg at 1050 °C (ISO 10694, 1995). To measure P and K concentration, 500 mg air-dry soil was passed through a sieve (2 mm) and afterwards extracted with 100 ml ammonium lactate–acetic acid buffer (pH 3.75) for 4 h at 200 rpm in dark polyethylene containers. After 1 h digestion of a 10.0 ml sub-sample of the filtrate with 3.0 ml hydrochloric acid (370 g/l) and 1.0 ml nitric acid (650 g/l) in a microwave oven (Milestone, ETHOS One, Sorisole Italy), the P and K concentrations were measured with ICP–OES spectrometer (Varian, Vista-pro axial, Palo-Alto, CA, USA).

Each of the four plots per block was divided into six sub-plots (6 × 7.5 m) and was assigned randomly to one of six annual N fertilization levels (0, 60, 90, 120, 150 or 200 kg N/ha per year) for the whole arable phase. The increasing N doses allowed the maize N response to be modelled, from which the N fertilizer replacement values (NFRV) of the different types of grassland could be calculated (see below). The highest fertilization level corresponded to the local legal threshold for N fertilization at that time. In each of the 3 years, the whole experimental field was limed with 2000 kg CaO/ha in March; ploughed, rotary harrowed and fertilized with 39 kg P/ha from triple superphosphate (206 g P/kg) and 205 kg K/ha from KCl (328 g K/kg) at the end of April. Afterwards, each sub-plot was fertilized manually with the appropriate amount of mineral N from calcium ammonium nitrate (270 g N/kg). Finally, the whole field was sown with an early maturing forage maize variety (cv. LG 32.20) at the end of April/beginning of May at a density of 114 000 seeds/ha (Table 4). The insecticide carbofuran (600 g/ha) was applied at sowing of maize in spring 2008 to avoid problems with wireworms (*Agriotes* sp.) in the first year of the arable phase. Weed management was equal to that applied on the PA plots during the arable phase.

At the end of the growing season (Table 4), the central 6 m^2 of the maize sub-plots were harvested manually. The stalks (with leaves and husks) and ears were weighed separately. The stalks were chopped, and a sample of c. 500 g was dried (16 h at 80 °C). A sample of ten ears was also dried (16 h at 80 °C and 4 h at 105 °C). The DMY represented in the Results section is the sum of ear and stalk DMY.

Table 4. Dates of sowing, harvesting and soil sampling for residual nitrogen of forage maize grown in the years 2008–2010

	Year		
	2008	2009	2010
Sowing date	8 May	24 April	27 April
Harvest date	24 September	10 September	1 October
Date of soil sampling	21 October	20 October	15 October

After harvest, soil samples were taken to determine residual mineral N concentration (Table 4). In 2008 and 2010, the soil was sampled up to a depth of 0.9 m. It was not possible to sample any deeper than 0.6 m in 2009 due to severe drought in September. As funds were insufficient to sample and analyse all sub-plots in every year, samples were taken on the sub-plots receiving 0 kg N/ha in 2008 and on the plots receiving 150 kg N/ha in 2009 and in 2010. These were considered as the lowest N fertilizations allowing maximum maize yield in the first, second and third years, respectively, after ploughing out grass swards, according to results obtained by Nevens and Reheul (2002). Using a gauge auger, four random spots were sampled per sub-plot and the samples bulked per sub-plot. Soil samples were extracted with 1 N KCl (extraction ratio 1:2) and the extracts were analysed for NO_3^- and ammonium (NH_4^+) with a continuous flow auto-analyser (Skalar, System 4, Breda, The Netherlands). To ease the comparison and the interpretation of the results, the amounts of NO_3^- and NH_4^+ in the soil were expressed in kg N/ha; where 1 kg of NO_3^- and NH_4^+ correspond to 0.226 and 0.776 kg N, respectively.

Two sub-plots were excluded from the trial for the years 2009 and 2010 because they were damaged by an excavator during the building of a wind energy converter on a site near the experiment. Both sub-plots were former PGg receiving 90 and 120 kg N/ha, respectively.

Data analysis

The experiment was analysed as a complete block split-split-plot design, with three blocks (Fig. 1). The three different ploughed-out grassland types and the arable land were the main plot factor (LEY), and the six different N fertilization levels were the sub-plot factor (*N*). Within each sub-plot, repeated measures were taken over three different years (YEAR). A linear mixed model was fitted to the data using the lmer() function in R (R Core Team, 2013). The unbalanced split-split-plot design (two sub-plots missing in years 2009 and 2010) was acknowledged using random-effect modelling: the blocks, main plots and sub-plots were added as nested random effects. To avoid large differences in magnitude between the predictors, N was rescaled by a factor of 0.1 for model fitting and the estimates reported in this manuscript are based on this rescaled variable. The effect of N on the maize yield was estimated using a quadratic model (Cerrato and Blackmer, 1990). Furthermore, three-way interactions of both the linear and quadratic N effects with YEAR and LEY were allowed.

The full model was simplified using a backwards selection procedure with type III analysis of variance (ANOVA) tests. For the model selection procedure, models were fitted using maximum likelihood. After the final model had been selected, the model was refitted using restricted maximum likelihood for correct estimation of the variance parameters in the mixed model. The N effects within LEY × YEAR strata were interpreted using simultaneous Wald tests (Bretz *et al*., 2010). Using the final model after model selection, the hypotheses of interest were tested by building specific contrasts derived from linear combinations of the fixed-effect model parameters, comparing estimated mean differences in DMY between groups of interest. An omnibus test across all contrasts of interest for both H1 and H2 was performed and post-hoc tests were performed after rejection of the omnibus test. A global type I error rate of 5% for both H1 and H2 was guaranteed in the post-hoc tests by constructing family wise error rate (FWER) adjusted *P* values and simultaneous 95% confidence intervals based on the multivariate normal approximation of the linear combination of parameters (Bretz *et al*., 2010). Hypotheses on individual parameters are assessed using likelihood ratio tests.

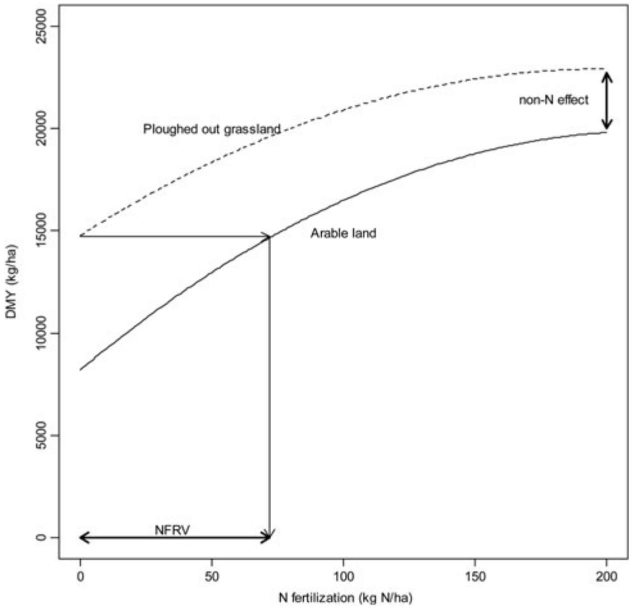

Fig. 2. Illustration of the determination of the nitrogen fertilizer replacement value (NFRV) and the non-N effect of ploughed out grassland using the maize yield response (yield data of 2009).

The N contribution effect of the ploughed-out leys to the following maize was calculated in two ways: using the NFRV method and using the difference method. The NFRV corresponds to the amount of mineral fertilizer that should be applied on permanent arable land to reach the yield found on unfertilized temporary arable land (Nevens and Reheul, 2002) (Fig. 2). It is the solution of the quadratic equation , where *a*, *b* and *c* are the coefficients of the model of the maize growing on PA in a particular year and *I* is the intercept (the maize DMY at 0 kg N/ha) of the model of the maize growing on the previous grassland type from which the NFRV is calculated in a particular year. The difference method is calculated as the difference between the economic optimal N fertilization rate (N_{opt}) on the temporary arable plots and the permanent arable plots. N_{opt} was defined as the point at which marginal fertilizer cost equalled marginal return from increased maize yield, calculated by using the first derivative of the N response curve (Lory *et al*., 1995). Forage maize dry matter was assumed to have a value of €88/ton and fertilizer N a value of €925/ton (Van der Straeten and Deuninck, 2016). This means that 1 kg

Table 5. Wald tests and restricted maximum likelihood parameter estimates of the model for the yield of maize growing on different ploughed grasslands (LEY) form 2008 till 2010 (YEAR) in function of the applied nitrogen (N, N^2)

	Estimate	Standard error	*t*-value
PA, year 2008 intercept	**10 273**	**683.0**	**15.0**
N			
1 D.F.; P < 0.001	**904**	**126.9**	**7.1**
N^2			
1 D.F.;P < 0.001	**−20**	**5.9**	**−3.4**
LEY			
3 D.F.; P < 0.001			
PGg	10 420	936.5	11.1
TGc	9426	934.8	10.1
TGg	9587	934.8	10.3
YEAR			
2 D.F.; P < 0.001			
2009	−2277	714.2	−3.2
2010	70	714.2	0.1
N:LEY			
3 D.F.; P < 0.001			
N:PGg	−837	167.2	−5.0
N:TGc	−564	166.1	−3.4
N:TGg	−671	166.1	−4.0
N:YEAR			
2 D.F.; P < 0.001			
N:2009	239	100.1	2.4
N:2010	162	100.1	1.6
LEY:YEAR			
6 D.F.; P < 0.001			
PGg:2009	2228	962.2	2.3
TGc:2009	−2723	949.1	−2.9
TGg:2009	−1755	949.1	−1.9
PGg:2010	−925	962.2	−1.0
TGc:2010	−6868	949.1	−7.2
TGg:2010	−5831	949.1	−6.1
N^2:LEY			
2 D.F.; P = 0.023			
PGg:N^2	21	7.7	2.7
TGc:N^2	6	7.6	0.8
TGg:N^2	12	7.6	1.6
N^2:YEAR			
2 D.F.; P = 0.021			
2009:N^2	−8	4.2	−1.9
2010:N^2	−10	4.2	−2.5
N:LEY:YEAR			
6 D.F. ; P < 0.001			
N:PGg:2009	−38	78.5	−0.5
N:TGc:2009	262	78.1	3.4
N:TGg:2009	155	78.1	2.0
N:PGg:2010	193	78.5	2.5
N:TGc:2010	319	78.1	4.1
N:TGg:2010	294	78.1	3.8

Note that estimates for each combination of YEAR and LEY involve linear combinations of the main effects and interactions. The reference class in the model corresponds to PA in the year 2008 (indicated in bold).

of fertilizer N should result in a DMY increase of at least 925/88 = 10.5 kg DM to be economically useful.

In case the maize DMY was no longer increasing with increasing N fertilization at the highest N level (200 kg N/ha), a non-N effect for the ploughed-out grassland was calculated as the difference between the maize DMY on PA and on the ploughed-out grassland (Fig. 2) (Johnston *et al*., 1994).

ANOVA was used to test the effect of LEY on the residual soil N in each of the 3 years: blocks were included in the models as a random factor.

Results

Maize dry matter yield

The three-way N^2:YEAR:LEY interaction effect was removed through model selection ($P = 0.11$). All remaining predictors were significant at $P < 0.05$. The final fixed-effect part of the mixed model was:

$$E(\mathrm{DMY}) \sim N \times \mathrm{LEY} \times \mathrm{YEAR} + N^2 \times \mathrm{LEY} + N^2 \times \mathrm{YEAR}$$

The estimators of the coefficients of the model can be found in Table 5.

There was a highly significant effect of N fertilization on the average DMY, which changed according to the year and management strategy combination (three-way interaction, $P_{\mathrm{N:LEY:YEAR}} < 0.001$; Fig. 3). Therefore, the N effects for all strata were interpreted separately. In general, an increase in the average yield in function of the N input was observed (linear term), which levelled off with increasing fertilization (quadratic term). In the first year, however, the pattern was only significant for the PA management strategy (FWER adjusted $P < 0.001$ and $P = 0.006$ for the linear and quadratic term, respectively). From the second year onwards, a significant positive linear N effect for all strategies was found (FWER adjusted $P = 0.012$), except for PGg in 2009. In the second year, the quadratic N effect was significant for the PA and TGc strategies (FWER adjusted $P < 0.001$ and $P = 0.003$, respectively), while in the third year, there was a significant attenuation for the PA, TGc and TGg management strategies (FWER adjusted $P < 0.001$ for PA and TGc, and $P = 0.017$ for TGg).

The fitted model was used to assess the hypotheses of interest proposed in the introduction using contrast analysis. The first

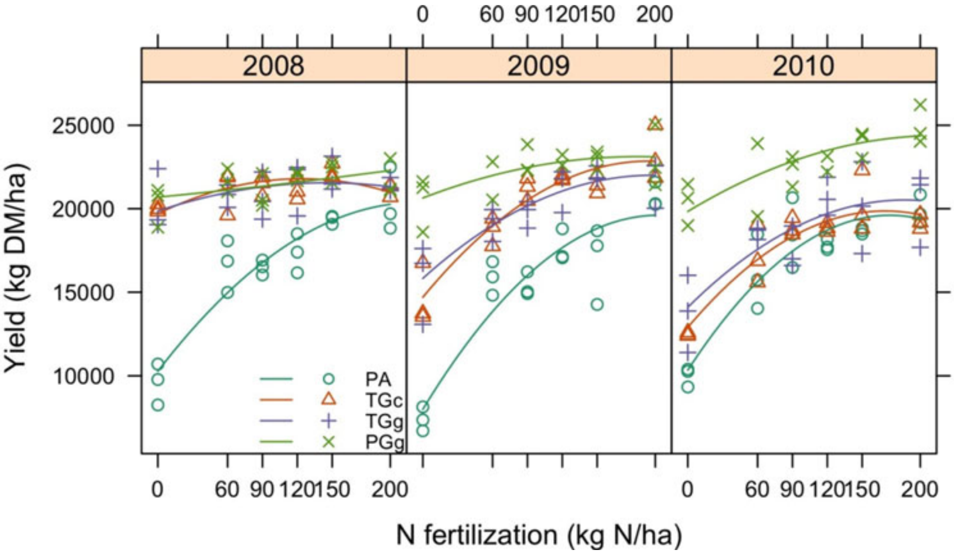

Fig. 3. Dry matter yield (DMY) (kg DM/ha) response to nitrogen (N) fertilization (kg N/ha) of forage maize grown on permanent arable land and on temporary arable land after ploughing out different types of grass–clover: PA, permanent arable land; TGg, grazed temporary grass–clover; TGc, cut temporary grass–clover; PGg, grazed permanent grass–clover. Maize yield was measured in the first (*a*); second (*b*) and third (*c*) year after ploughing out the grass–clover. The points represent the raw data and the lines represent estimated mean trends based on the mixed model. Colour online.

global hypothesis, assessing whether ploughed-out grazed temporary grass–clover delivered more N to the forage maize than cut temporary grass–clover, was non-significant. The second global hypothesis, which assesses whether ploughed-out permanent grass-clover (PGg) delivered more N and during a longer period than temporary grass–clover (TGg and TGc), was highly significant ($P < 0.001$). In 2008, no difference was found between temporary and permanent ploughed-out grass–clover, but starting from 2009, higher yields were observed for ploughed-out PGg on the plots with lower (0, 60 and 90 kg N/ha) N fertilization compared with both TGg and TGc (Fig. 4). This difference was even more pronounced in 2010, where a significantly ($P < 0.001$) lower yield was found on ploughed-out TG compared with PG across all N rates.

Nitrogen contribution effect and non-nitrogen effect

In all 3 years, the maize yield on PGg without any N fertilizer exceeded that of the highest fertilized PA plots, receiving 200 kg N/ha (Fig. 3). As a result, the exact NFRV could not be calculated, but it was at least 200 kg N/ha (Table 6).

For TGc and TGg, the NFRV decreased through the years. Overall, the NFRV of PGg was much higher than that of the TGg and TGc. In 2009 and 2010, the NFRV of TGg was slightly higher than that of TGc (Table 6).

The calculated N contribution effects, using the N difference method, were between 113 kg N/ha on TGc and 195 kg N/ha on PGg in 2008, between 22 kg N/ha on TGc and 77 kg N/ha on PGg in 2009, but decreased to values below 10 kg N/ha in 2010 for all types of former grassland.

For 2009, non-N effects of 2527, 1019 and 2552 kg DM/ha for TGc, TGg and PGg, respectively, were calculated. They corresponded to 0.125 and 0.12, respectively, of the maize yield on PA receiving 200 kg N/ha (20 715 kg DM/ha). For 2010, a non-N effect of 3628 kg DM/ha was calculated for the maize grown on PGg, corresponding to 0.18 of the maize yield on PA (19 898 kg DM/ha).

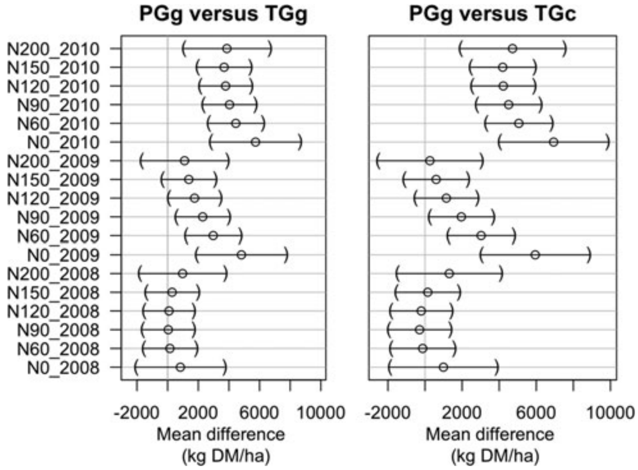

Fig. 4. Comparison of ploughed out permanent grass–clover (PGg) to temporary grass–clover (TGg: grazed and TGc: cut) on a global 5% significance level over all comparisons. The figure shows estimated mean differences in maize dry matter yield (points) and simultaneous 95% confidence intervals as interval around the estimated means for each comparison in the post-hoc analysis for all combinations of N fertilization (N0: 0 kg N/ha to N200: 200 kg N/ha) and years (2008–2010).

Residual soil nitrogen

In 2008, residual soil NO_3^- concentration was significantly ($P < 0.001$) higher on TGg plots (40 kg NO_3^--N/ha) and PGg plots (68 kg NO_3^--N/ha) than on PA plots (18 kg NO_3^--N/ha). In 2009 and 2010, PGg plots had a significantly ($P = 0.001$ in 2009; $P < 0.001$ in 2010) higher residual soil NO_3^- concentration than PA plots (Table 7). In 2010, residual soil NO_3^- on PGg plots was ten times higher compared with PA plots and exceeded the legal autumn threshold of 90 kg NO_3^--N/h at the trial location.

In 2009, residual soil NH_4^+ concentrations on TGg and PGg plots were significantly ($P < 0.001$) higher than on TGc and PA plots in 2009; in 2008 and 2010 on the other hand, there were no significant differences.

Table 6. The N contribution effect (kg N/ha) of ploughed leys in the first 3 years after ploughing out different types of grass–clover using the nitrogen fertilization replacement value (NFRV), the economic optimal N rate (N_{opt}) and the difference method

	NFRV			N_{opt}			Difference method		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
PA	–	–	–	195	183	155	–	–	–
TGc	182	71	21	82	168	146	113	15	9
TGg	172	87	40	78	161	155	117	22	0
PGg	>200	>200	>200	0	106	157	195	77	0

TGg, grazed temporary grass–clover; TGc, cut temporary grass–clover; PGg, grazed permanent grass–clover.

Table 7. Residual nitrate (NO_3^-) and ammonium (NH_4^+) nitrogen (kg N/ha) in the soil (0–0.9 m in 2008 and 2010, 0–0.6 m in 2009) after the harvest of forage maize grown on permanent arable land and on temporary arable land after ploughing out different types of grass–clover in the years 2008–2010

	Year (N fertilization level)								
	2008 (0 kg N/ha)			2009 (150 kg N/ha)			2010 (150 kg N/ha)		
	NO_3^-	NH_4^+	N	NO_3^-	NH_4^+	N	NO_3^-	NH_4^+	N
Permanent arable	18	25	43	21	6	27	16	23	39
Cut temporary grass–clover	28	27	55	26	7	33	27	20	47
Grazed temporary grass–clover	40	26	66	37	31	67	46	17	63
Grazed permanent grass–clover	68	26	94	59	25	84	166	19	185
P-value[a]	***	0.936	**	**	***	***	***	0.091	***

[a] Results of ANOVA with LEY as fixed factor (D.F. = 3) and BLOCK as random factor (D.F. = 2).
$^{**}P < 0.01$; $^{***}P < 0.001$.

Discussion

Experimental design

For historical and practical reasons (time), an existing PG sward with adjacent arable land was used to build the current experiment. Therefore, the ploughed-out permanent grass–clover (PGg) land plots were not randomized but all located on the western side of the experimental field, which might have caused confounding between potential 'column effects' (caused by the fixed location of the PGg plots) and effects of the PGg management strategy. There are, however, no indication that the soil on which the PGg was installed was of a different quality to the soil on which the remainder of the experiment was established: the piece of land on which the current experiment was established is the most uniform, highest yielding block of land present on the experimental farm: it is drained and absolutely flat. Moreover, the modest size of the experimental area (0.32 ha) makes large spatial heterogeneity unlikely. This statement is supported by the absence of column effects in the DMY measured on the TG and PA plots. Also, note that the effects of N on the DMY within a management strategy will not be confounded given that there is no interaction between N and location, which is a general assumption in the analysis of complete block designs.

Forage maize dry matter yield after ploughed-out grass

The current results confirm that ley-arable rotations with forage maize have the potential to obtain high yields with reduced fertilizer N. The management (cutting or grazing) of the grass–clover swards during the ley phase did not lead to yield differences in the following maize crops, which is in line with former studies focusing on spring cereals (Eriksen, 2001). The clover present in swards and reduced N fertilization on the grazed swards apparently compensated for the difference in N balance as a result of the faeces and urine recycling on the grazed leys (Vinther, 1998; Vertès *et al*., 2015). Support for this hypothesis can be found in the study of Eriksen (2001): in a 3-year ley–3-year cereals rotation, a different effect of management (grazing *v*. cutting) was found in pure grass leys but not in grass–clover leys in the first year after ploughing. After ploughing pure ryegrass swards, the NFRV was 90–100 kg N/ha on grazed swards compared with 25 kg N/ha on cut swards whereas after ploughing out ryegrass–white clover swards, there was no effect of grazing on the NFRV.

There was a clear effect of sward age on maize performance following ploughed-out grass–clover: in the third year after ploughing, significantly higher maize yields were found in permanent grass–clover compared with 3-year-old temporary grass–clover. In the study of Christensen *et al.* (2009), which focused on the age effect of 1–6-year-old ploughed-out grassland on spring barley yield, the positive effect of grassland age on the yield was limited to the first year of the arable phase in the absence of N fertilization. The increased age difference in the current trial, almost 40 years between PG and TG, reflected for instance in SOC content 1.7 times higher in PG, can explain why the effect in the current trials lasted longer compared with Christensen *et al.* (2009).

The age effect of the ploughed-out grassland can be partly explained by the SOM dynamics after ploughing. In the first year after ploughing out grass–clover, the major part of released N comes from decomposition of fresh plant residues. The amount of N contained in the ploughed-out fresh plant residues can easily be as high as 200 kg N/ha (Vertès *et al*., 2007). There seems to be little or no effect of sward age on the amount of ploughed-out biomass and its N concentration. Christensen *et al*. (2009) compared with 3- and 6-year-old grass swards receiving 225 kg N/ha per year and found no significant difference in root + shoot biomass (11.7 *v*. 12.6 t DM/ha, respectively) or in the corresponding N contained in this biomass (150 *v*. 176 kg N/ha). The data of the current study correspond with these observations: in the first year (2008), N fertilization only had a significant effect for permanent arable land, while the other management strategies benefited from mineralization of ploughed-out leys, resulting in a non-significant effect of N fertilization.

From the second year on after ploughing out grassland, N originates mainly from decomposition of remaining plant residues (larger roots, stubble) and SOM. The importance of the former source of N decreases markedly with time (Vertès *et al*., 2007). In the current study, the effect of ploughed-out TG on maize yield decreased greatly from the second year on, while there still was a clear positive effect on maize DMY of ploughed-out PG. This indicates that from the second year on, the effect of decomposition of plant residues incorporated in the soil decreased greatly and the effect of SOM mineralization increased gradually. This explains the longer and greater effect on maize yield of ploughed-out PG (with a high SOM concentration) compared with the ploughed-out TG with modest SOM concentration. Taking into account the soil bulk density of 1450 kg/m^3, assuming a C/N ratio of the SOM of 10 and a mineralization coefficient of 2% (Sleutel *et al*., 2006), the calculated yearly N mineralization from SOM in the 0–0.3 m top soil layer was 87 kg N/ha on TG plots (10 g SOC/kg soil) and 148 kg N/ha on PG plots (17 g SOC/kg soil), or a difference of 61 kg N/ha. In the third year of the arable phase, N fertilization of c. 100 N on TG plots was required to obtain the yield level of PG plots at 0 N. Taking into account the differences in mineralization between PG and TG calculated above, a share of 0.6 (61 kg N/ha of this 100 kg N/ha) of the difference in yield on PG and TG at 0 kg N/ha can be explained by the difference in mineralization from SOM.

So, the remaining 0.4 share of this yield difference between PG and TG can be explained by a non-N effect (Johnston *et al*., 1994; Nevens and Reheul, 2002) or a 'soil fertility effect' as was suggested by Piutti *et al*. (2015): better water holding capacity, better soil structure, higher cation exchange capacity. In the second and third year of the arable phase, the shares of total yields of the non-N effects of ploughed out PG were 0.12 and 0.18, respectively, of the yield on PA with N fertilization of 200 kg N/ha.

Nitrogen fertilization after ploughed-out grassland

The NFRVs in the first 3 years after ploughing out the grass-clover swards were around 177, 79 and 31 kg N/ha for TG, respectively (averages between TGc and TGg) and >200, >200 and >200 kg N/ha for PG. Corresponding economical N rates (N_{opt}) were 80, 165 and 150 kg N/ha, respectively, for TG and 0, 106 and 157 kg N/ha for PGg; values for PA were 195, 183 and 155 kg N/ha. Using the difference method, the N contribution effects of the ploughed-out leys during three consecutive years were 115, 18 and 5 kg N/ha, respectively, for TG and 195, 77 and 0 for PG.

The non-N effect was clearly visible: in the third year of the arable phase, the yield response functions of PG and PA were virtually parallel, meaning that the response to N was similar for both. The function for PG, however, is situated above that for PA, which means that the yield on PG is higher than that on PA at any N fertilization: this yield difference corresponds to the non-N effect. Similar but smaller non-N effects can be seen in the second year of the arable phase for the maize growing on former TG.

Lory *et al*. (1995) suggested basing N fertilization recommendations in practice on the N_{opt} method rather than the NFRV method. Based on N_{opt}, N fertilization is higher, but the corresponding yield is also higher. In the current study, for TGc in 2009, for example, the NFRV method recommends 71 kg N/ha, which results in a maize yield of 19 577 kg DM/ha. The N_{opt} method, on the other hand, suggests fertilizing with 168 kg N/ha, resulting in a maize yield of 22 705 kg DM/ha. The extra 97 kg N/ha provokes a marginal response of 32 kg DM/kg N, three times as much as the calculated economical return. A disadvantage of the N_{opt} method is that the values calculated depend on fertilizer price and the value of forage maize DM, which are market-driven and fluctuate over time.

The current findings confirm the findings of Nevens and Reheul (2002) and Kayser *et al*. (2008), who concluded that maize can express its full yield potential without N fertilization in the first year after ploughed-out grassland swards at least 3 years old. From the economic point of view, N fertilization of around 80 kg N/ha would be justified after TG. From the second year on, N fertilization was needed to optimize maize DMY on temporary arable land, but N_{opt} rates after ploughed-out grassland were lower and yields higher compared with permanent arable land. In the third year, N_{opt} rates calculated for the ploughed-out grassland and permanent arable land were almost equal, but owing to the non-N effect, maize yields continued to be about 20% higher after ploughed-out PG.

Soil mineral nitrogen and nitrogen-leaching on ploughed-out grassland

Although residual N was mostly higher on the TG and PG plots compared with the PA plots, the values found on TGc (33–55 kg N/ha) and TGg (63–66 kg N/ha) can be considered normal under the trial circumstances. Indeed, based on a meta-analysis of field trials in Belgium, D'Haene *et al*. (2014) calculated an average residual soil mineral N of 61 kg N/ha after maize receiving 150 kg N/ha.

No difference in residual soil N between TGc and TGg was found in the current study. This is in accordance with Eriksen (2001): after ploughing out 3-year-old unfertilized grass–clover that was either cut or grazed by cows receiving 130 or 260 g N/day from concentrates, NO_3^- leaching in spring barley was more influenced by fertilization than by grazing or cutting management. One might argue that the applied N dressings were different on TGc and TGg, but this was justified since Eriksen *et al*. (2010) confirmed that a substantial part (>70%) of the N ingested by the grazing animals is recycled through excreta. From this excreta-N, an unknown proportion is lost through volatilization and leaching.

Compared with TG plots, the residual soil mineral N was greater on PG plots: 94, 84, 185 kg N/ha in the first 3 years of the arable phase, respectively. In the third year, the values for residual NO_3^--N exceeded the local legal limit (90 kg N/ha in the period 1 October till 15 November in the 0–0.9 m soil layer), for an unknown reason.

There is a greater risk of increased NO_3^- leaching upon ploughing out permanent grass–clover, but there are a number of preventive and curative means to cope with this. Long-lasting permanent grassland should be ploughed out only exceptionally; therefore, one should care for it by all means in order to keep it permanent. If at last renewing is necessary, losses may be limited by applying good agronomic practices: destruction of the sward in spring rather than in autumn (Djurhuus and Olsen, 1997; Seidel *et al.*, 2004); appropriate N fertilization (Kayser *et al.*, 2008); use of cover crops in the autumn/winter after harvesting the main crop (Vos and Van Der Putten, 1997; Hansen *et al.*, 2007) and growing a main crop with high N demand and export, such as fodder beet, in the first year after ploughing (Nevens and Reheul, 2002).

The risk of N leaching and groundwater pollution in ley-arable rotations with forage maize should be assessed in the perspective of the entire rotation. The potentially increased risk of N leaching in the arable phase of ley-arable rotations may be compensated by lesser N leaching in the ley phase. Recent research by D'Haene *et al.* (2014) showed that silage maize and grassland grown with the maximum allowed effective N fertilizer application rate on loam soils in Belgium resulted on average in a residual soil mineral N of c. 60 and 30 kg N/ha, respectively. Therefore, if the ley-arable rotation replaces maize monoculture, the potentially greater N leaching in the arable phase is likely to be compensated by the lesser N leaching in the grassland phase. A similar result has been reported by Webster *et al.* (1999), who estimated N losses by leaching over the whole period of a 5-year ley-arable rotation with winter cereals. Leaching was estimated at 17 kg N/ha per year in the ley-arable system compared with 29 kg N/ha per year and 2 kg N/ha per year for an all-arable system and continuous grass, respectively.

Conclusion

Referring to the hypotheses (H) put forward in the Introduction section, the following conclusions can be made:

(H1) There was no difference in the N contribution effect of ploughed-out temporary grazed grass-clover compared with temporary cut grass–clover. The NFRV for ploughed-out temporary grass–clover were around 180, 80 and 30 kg N/ha in the first 3 years, respectively.

(H2) The effect on maize DM yield of ploughed-out permanent grass–clover was greater and longer lasting than that of temporary grass–clover. Using NFRV, the calculated N contribution effects were greater than 200 kg N/ha in each of the three consecutive years after the ploughing out of the permanent grass–clover. The non-N effects lasted longer than the N effects: in the third year of the arable period, the non-N effect of ploughed permanent grass–clover was 18% of the DM yield on permanent arable land.

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