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*** Revision Notes**

Eduard Hovens
Journal Manager
Agriculture, Ecosystems and Environment

10 January 2006

Dear Eduard Hovens,

Thank you for your mail dated 11/24/2005, where you subject to satisfactory minor revision accepted our paper ”Nitrate leaching and breadmaking quality….” (AGEE892). Needless to say, we are very pleased about your decision.

We have considered the points raised by you and the two reviewers, which have been very helpful in improving the manuscript. The response to these points is listed on the next pages.

Yours sincerely

Jørgen Eriksen

Response to editor's and reviewers' reports

Editor

- The conclusion section is now a separate section.
- The unit in Table 1 is now in parenthesis.
- We believe that the Fig. 2 legend SE should not have +/- attached to it?
- Fig. 5 has been revised and improved in print quality.

Reviewer 1

- p3 line 22: Sand and silt percentages has been included
- p3 line 26: Plot size has been included. Areas were the same in all leys.
- p4 line 3: Cattle slurry treatments were applied to three subplots of 12x12 m within the grassland plots. This has been clarified.
- p4 line 19: The procedure was only repeated in the unfertilized plots as we here had the "pure" residual effect unbiased by fertilizer application. This is now explained in the text.
- p5 line 23: A reference has been included.
- p5 line 27-30: The word "grazed" does not make sense here – it has been deleted. We have experienced that three cups per plot and four replicates giving a total of 12 cups is sufficient in most cases. The reference to "three or four replicates" was wrong. "three cups" has replaced it.
- p7 line 2-9: The root biomass is now compared to literature.
- p8 line 1-7: The difference between the two precrops is actually commented on earlier in section 3.2. in the sentence "Grain yield and N uptake were significantly higher following ryegrass ($P<0.05$ and <0.01, respectively) than following grass-clover."
- p8 line 11-17: The cereal precrops are thoroughly commented on in the general discussion (second paragraph), and we prefer to keep it there.
- p8 line 21: Further explanations to the methods are included.
- p8 line 21: Fig. 5 has been improved in print quality.
- p11 line 3-10: The leaching data has been put in context to other published work.
- p13: The conclusion is now more balanced in relation to bread making quality.

Reviewer 2

- Material and methods, Rheology: Small deformation rheological methods were used to discriminate between samples with minor differences in rheological properties due to the effect of the growth conditions. Earlier studies have shown that these methods are suitable for these purposes. In the present work, the Farinograph was only used in the baking tests to obtain equal dough consistencies, as water was added to 500 BU on the recorder. These values of water absorption have been included in the multivariate data analysis and in the discussion of the results.
- Baking experiment: The description of the baking test has been extended and more details are included.
- Results, p9 line 30 to p10 line 22: The PCA and PLSR are revised with the water absorption included. A new figure of the last PLSR-model for the grass-clover is included together with comments to the plots.
- Results, p10 line26 to p11 line 16 and figure 7: Nitrate leaching was not determined with a cereal history.

- Results p11 line 6-8 and figure 7: It is tempting to say that the lack of significance regarding fertiliser application was caused by a large variability within ryegrass. However, when performing the statistical analysis on grass-clover only, the effect of fertiliser is still insignificant (P<0.18). Considering this and the fact that we do not know the origin of the variability in ryegrass, we have decided not to include this in the text. Please let us know if you think otherwise.
- Results p11 line 27: The word ”en” has been changed to ”an”.
- The paragraph has been more clearly expressed as indicated by the referee.

Nitrate leaching and bread making quality of spring wheat following cultivation of different grasslands

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Abstract

The influence of sward botanical composition and ley age on grassland residual effects, quality of spring wheat and subsequent nitrate leaching was investigated. Grazed grasslands of different age (1, 2 and 8 production years) and composition (unfertilised grass-clover and fertilised perennial ryegrass) were ploughed and followed by spring wheat and spring barley. For reference, an adjacent field without grassland history but with the same crop sequence in 2002-2003 was treated with increasing quantities of N fertiliser. Yields and N uptake of spring wheat following grasslands always exceeded those of the reference plots with a history of cereal production. The nitrogen fertiliser replacement values of grass-clover and ryegrass were 59-100 and 72-121 kg ha^{-1}, respectively, with the highest values representing the 8-year-old leys. Grain yield and N uptake increased while those for straw decreased with increasing ley age. There were no effects of previous grassland type (grass-clover/ryegrass) on content of protein, starch and gluten, but grassland age significantly influenced protein ($P<0.05$) and gluten ($P<0.01$) contents. It is suggested that N mineralisation following the ploughing of older grass leys occurred later than when following the 1st year ley. The protein and gluten contents of wheat following unfertilised grass-clover corresponded to those following cereals given 125-150 kg N ha^{-1}, but the rheological properties of the gluten were different to what could be achieved using equivalent quantities of mineral fertiliser. Probably, the slow release of N from decomposition of old grassland gave a better synchrony between N release and plant demand. Nitrate leaching in year 1 after ploughing was significantly influenced by type of grassland ($P<0.001$) with 10 and 29 kg N ha^{-1} leached from grass-clover and ryegrass, respectively. Nitrate leaching following ploughing of 1-year-old leys averaged 11 kg N ha^{-1} which was significantly lower than the 24 kg N ha^{-1} following 2 or 8-year-old leys. The flow-weighted mean nitrate concentration decreased from 8.5 mg N l^{-1} in year 1 after grassland cultivation to 4.5 mg N l^{-1} in year 2. More N was released following ploughing of ryegrass swards and from grasslands of increasing age, but differences were moderate compared to the estimated N-surplus. This indicates that when organic matter in grasslands is partially decomposed and readily mineralisable N used, the remaining organic N is released only very slowly.

Keywords: Bread-making quality; Grassland cultivation; Grass-clover; Nitrate leaching; Residual effect; Ryegrass

1. Introduction

Grazed grasslands accumulate N as most of the N-intake by ruminants is excreted and thus recycled to the field during grazing (Whitehead, 1995). Mineralisation rates in grasslands also tend to be low compared to arable crops where tillage operations disturb and expose soil organic matter to rapid oxidation (Dao, 1998; Eriksen and Jensen, 2001). The extent of N build-up in grasslands depends on fertilisation regime, livestock feeding practices, stocking density, time of grazing, the botanical composition (Cuttle and Scholefield, 1995) and sward age (Johnston et al., 1994). As a consequence, the ley/arable crop rotation carries a high risk of nitrate leaching especially during the period following ploughing of grasslands when there is a rapid mineralisation of N, often exceeding the requirement of the succeeding crop (Francis, 1995). To avoid the adverse environmental impact of nitrate leaching (Addiscott et al. 1991) it is of utmost importance to utilise the N from grasslands as efficiently as possible.

It has been shown that good management practices following grassland cultivation may control nitrate losses. This includes delaying ploughing until late winter or spring (Francis et al., 1992; Djurhuus and Olsen, 1997), the use of efficient catch crops after ploughing (Francis, 1995) and a reduction in fertiliser N application to cereals after ploughing (Eriksen, 2001; Nevens and Reheul, 2002; Vinten et al., 2002). The release of substantial quantities of N from grass-clover residues means that fertiliser-N application to subsequent cereals can be reduced or even eliminated in the first following crop (Watson et al., 2005). However, in order to reduce N fertiliser input to cereals following grass without losing biomass, it is necessary to determine more precisely what controls the quantity of N released following ploughing. This is especially relevant for crops like bread wheat that depends on nitrogen availability for optimal baking quality.

It is well known that baking quality of wheat is related to the quantity and quality of the gluten protein in the wheat grain. The glutenins and gliadins, which are the main components of the gluten proteins, both have a great impact on the dough properties and the form and volume of the loaf. (Gupta et al., 1992; Magnus et al, 2000; Vereijken et al., 2000). Differences in gluten composition are related to the genetic background and also to environmental factors such as N availability (Luo et al., 2000; Peltonen and Wirtanen, 1994; Wooding et al., 2000) and N application have been shown to increase the gliadin content (Daniel and Triboi, 2000; Johannson et al., 2001; Tronsmo et al., 2003).

The objectives of this study were to determine grassland residual effects, quality of spring wheat and the effect of botanical composition (grass clover/ryegrass) and ley age (1, 2 and 8 years) on nitrate leaching.

2. Materials and Methods

2.1. Field experiment

The experiment was located at the Burrehøjvej experimental field at Research Centre Foulum in the central part of Jutland (9°34'E, 56°29'N). The soil is classified as a Typic Hapludult with 8% clay, 29% silt, 57% sand and 3.6% C. More soil properties are given in Eriksen (2001). In 2001 the experimental plots represented three grazed grasslands of different ages (1, 2 and 8 production years) (Fig. 1) and two different pre-crops: unfertilised grass-clover (perennial ryegrass [*Lolium perenne* L.]/white clover [*Trifolium repens* L.]) and fertilised perennial ryegrass (300 kg N ha^{-1} yr^{-1}). The swards were arranged in a block design with four replicates (Fig. 1). These plots, that were 576 m^2 each, and the surrounding grassland were grazed by a herd of dairy cows to maintain a grass height of approx. 6 cm.

In late March 2002 all plots were rotovated to 6-8 cm depth and ploughed 11 days later to 20-22 cm. The crop sequence following ploughing of the swards was spring wheat and spring barley in 2002 and 2003, respectively, both years with perennial ryegrass undersown as a catch crop (8 kg seed ha^{-1}). In the spring of 2003 catch crops were incorporated in the same way as the original swards.

Fertiliser was applied to three subplots (12x12 m) within each grassland plot. Fertiliser treatments were 0, 115 or 230 kg total-N ha^{-1} in cattle slurry (c. 55% NH_4-N) using trailing hose application prior to ploughing and sowing of the cereals. To estimate pre-crop effects, triplicate plots (3x12 m) with no grassland but a cereal pre-crop history were set up as reference in an adjacent field. The same crop sequence as main plots was followed in 2002-2003 and the plots were treated with increasing quantities of N fertiliser (0, 25, 50, 75, 100, 125 and 150 kg N ha^{-1} as calcium ammonium nitrate) and with 32 kg P and 168 kg K ha^{-1} in a PK-fertiliser. Apart from the fertiliser used in the reference area, organic farming practices (no mineral fertiliser and pesticides) were adopted for the cereal crops.

2.2. Plant and soil sampling

Immediately before the start of soil tillage operations, roots and tops of each sward type were sampled. In each sward 16 soil cores (52 mm diameter) were taken to a depth of 20 cm for determination of root biomass (Eriksen and Jensen, 2001). For determination of corresponding aboveground biomass, grass tops were sampled in two 0.25 m^2 areas in each plot by cutting at the soil surface. Dry matter, C and N contents were determined in all root and top samples. The procedure was repeated in the unfertilised plots in spring 2003 before incorporation of the ryegrass catch crop. Only unfertilised plots were used in 2003 to estimated the residual effect unbiased by fertiliser application.

In late August 2002, all spring wheat plots were harvested in an area measuring 6x6 m using a plot combine. In mid July 2003 barley was harvested as a wholecrop. In the adjacent field without grassland history an area of 1.5x10 m was used for harvesting. Sub-samples were taken for determination of DM and N content.

2.3. Determination of bread-making quality

Grain samples from each plot were analysed for dry matter, protein and starch content (w/w %) in the laboratory using a near infrared spectroscopy analyser (Foss Tecator, Infratec

1241). The near-infrared spectroscopy analyser was calibrated and linked to the Danish NIT network (Buchmann et al., 2001).

Baking quality was characterised for spring wheat following cereals and grass-clover. Plots from cereals were analysed separately (three replicates), whereas plots from grass-clover were mixed. This resulted in two replicates of each grass age, and six replicates of each fertiliser treatment.

Grain samples were stored for four months before milling using a Brabender Quadromat Junior® mill. Glutens were isolated from the flour in an automated gluten washer (Glutomatic 2100, Perten Instrument) according to ICC (1996). Gluten content was determined as % w/w of flour. Flour protein was determined as Nx5.7 (Dumas). Gluten washing was done in duplicate. After isolation, the glutens were loaded into a controlled stress rheometer (Bohlin, CVO), and rested for 5 min before measurements were started. Parallel plate geometry with a diameter of 25 mm and a gap of 2 mm was used for the rheological characterisation, which included creep recovery and oscillation measurements. The parameters from creep recovery were maximum strain after 5 min of creep (creep), recovery strain after 5 min of recovery (recovery), and the relative recovery (% recovery). From oscillation measurements storage modulus (G'), loss modulus (G''), and phase angle (δ, $\tan^{-1}G''/G'$) at 1 Hz and 0.01 Hz were used to characterise the elastic and viscous properties of glutens. Rheological measurements were repeated twice.

Bread-making quality was determined using a small-scale baking test. The dough was prepared from 100 g flour, 1.67 g sugar, 1.67 g salt, 4.44 g shortening, and 5.55 g pressed yeast by mixing at 126 rpm in a Farinograph bowl. Water at 30 ^{0}C was added during the first two minutes of mixing together with 6 mL 1% (w/w) ascorbic acid solution, and the mixing time was 7 min. After mixing 150 g of dough was moulded on the Extensograph, and the loaf was placed in a proving cabinet at 38 ^{0}C and 80 % RH. After 45 min of proving the loaf was baked at 210 ^{0}C for 19 min. Baking tests were done on three samples from each N-application including three grass ages. Loaf volume was determined by displacement of rapeseeds, and this result used as the indicator of bread-making quality (Tronsmo et al., 2003).

2.4. Nitrate leaching

Before the start of the experiment, three ceramic suction cups were installed in each of the subplots at a depth of 1 m and 2 m apart. Every two weeks a suction of approximately 80 kPa was applied three days prior to sampling. The samples were analysed separately or bulked with equal sample volumes from each of the three cups per subplot before analysis of nitrate concentrations by the method of Best (1975). The nitrate determination also included nitrite, which was assumed to be negligible.

The water balance was calculated using the model Evacrop (Olesen and Heidmann, 1990) for which inputs were daily meteorological measurements (precipitation, temperature and evaporation) and crop type, time of sowing and soil physical parameters. Nitrate leaching was estimated using the trapezoidal rule (Lord and Shepherd, 1993), assuming that nitrate concentrations in the extracted soil water represented average flux concentrations. The accumulated leaching was calculated from the beginning of April to the end of March.

2.5 Data analysis

Dry matter yields and annual nitrate leaching were analysed as a split-split-plot experiment with sward type as main plot factor, cropping sequence as subplot factor and fertiliser application as sub-subplot factor using the MIXED procedure of the SAS statistical package. The analysis of nitrate leaching was made on log-transformed data to obtain homogeneity of variance, but results in figures are presented as arithmetic means with S.E.

Principal component analysis (PCA) and partial least squares regression (PLSR) were carried out using Unscrambler (version 7.8, CAMO, Norway). With PCA it is possible to study the variability among several variables, which are projected down on a few latent variables - the principal components (PCs). In PLSR the PLS factors are estimated to describe the covariance between a set of x-variables and y-variables. In the present work PCA was performed to study the variability in the quality characteristics and the samples, which are represented by the loading plot and the score plot, respectively. PLSR was used to study the relationships between protein and gluten content, rheological characteristics and the loaf volume.

3. Results and Discussion

3.1. Incorporated plant residues

The different aged swards were incorporated in spring 2002 and the ryegrass catch crop the following spring. Isolation of plant material in spring is difficult and especially sampling of roots was subject to great uncertainty with coefficients of variation of up to 47% (Table 1). In 2002 both DM and N contents increased with increasing sward age ($P<0.05$). There was no difference in DM content in ryegrass and grass-clover swards but the N content was significantly higher in ryegrass ($P<0.05$). The quantities of plant residues were higher than found in some studies (e.g. Hauggaard-Nielsen et al*.,* 1998; Høgh-Jensen and Schjoerring, 1997), which may be due to a higher age and more intensive use of the present pastures, but also soil type may be of importance as similar quantities were found by Eriksen (2001) on this soil. In 2003 there were no longer any effects of the different prior uses on the catch crop plant materials. The undersown catch crop was well established with good ground cover in the autumn. However, the DM and N levels in the catch crops were much lower than in the previous grasslands. On average 181 kg N ha^{-1} was found in the grassland swards compared with only 78 kg in the catch crop (the cereal reference not included). These changes were mainly caused by root DM decreasing much more dramatically than top DM. At the initial ploughing, the root-to-top ratio was on average 13 compared with only 3.1 in the following year.

3.2. Yield and residual effect

The residual effect of grasslands may be estimated from plant production in unfertilised plots (Fig. 2). Yields and N uptake of spring wheat following grasslands always far exceeded those in the reference plots with a history of cereal production. Thus, N uptake in unfertilised plots following grassland was 46-70 kg ha^{-1} higher than in plots following cereals. Grain yield and N uptake were significantly higher following ryegrass ($P<0.05$ and <0.01, respectively) than following grass-clover. Both straw and grain yields and N uptakes were affected by ley age at the time of ploughing ($P<0.01$ and <0.001, respectively) but in opposite directions. Grain yield and N uptake increased while those for straw decreased with increasing ley age.

Differences were especially marked between 1- and 8-year-old leys. As an average of ryegrass and grass-clover, the N uptake in grain increased from 84 to 99 kg ha^{-1} and N uptake in straw decreased from 55 to 41 kg ha^{-1} without significant effects on total N uptake. These differences in plant N partitioning indicate differences in the timing of N mineralisation as late application of N to spring wheat has been shown to increase the N content in grain (Petersen, 2004). From these findings it may be suggested that N mineralisation following the ploughing of older grass leys occurred later and in better synchrony with crop demand than with 1^{st} year leys.

The nitrogen fertiliser replacement value (NFRV) of grasslands may be estimated from the yield response curve for mineral fertiliser application to spring wheat following a cereal cropping history (Fig. 3). On a grain yield basis, NFRV of grass-clover was 59-100 kg ha^{-1}, with the highest value representing the 8-year-old ley, and NFRV of ryegrass was 72-121 kg ha^{-1}, similarly with the highest value following 8-year-old leys.

The considerable residual effect of grasslands is further emphasised by the lack of significant spring wheat grain yield response to fertiliser application following grassland ploughing (Fig. 3). Higher yields following 8-year-old leys compared with 1 and 2-year-old leys irrespective of fertiliser application were probably caused by the non-nitrogen effects of older grasslands such as improved soil structure and better resistance to fungal diseases. In year two following grassland ploughing both yield and N uptake responded significantly to fertiliser application ($P<0.001$).

3.3 Baking quality

Grain samples following grass-clover and cereals were analysed for content of protein, starch and gluten using near infrared transmittance spectroscopy (Fig. 4). There were no effects of previous grassland type (grass-clover/ryegrass), but grassland age significantly influenced protein ($P<0.05$) and gluten ($P<0.01$) contents. The percentage of protein and gluten was higher following 8-year-old leys than one-year-old leys. All parameters (protein, starch and gluten) were strongly affected by fertiliser application ($P<0.001$). Protein and gluten contents increased and starch content decreased with increasing application rates.

Variability in the composition of the gluten proteins will be reflected in the rheological properties of the gluten network. Rheological measurements at low shear strains are nondestructive, and thus provide information of the viscoelastic properties of the glutens in terms of stress and strain. The methods can be transient as creep recovery, thus providing information of long-time behaviour of the dough, or dynamic as oscillation, which imply stress in a sine wave at different frequencies. These methods have been used extensively to study gluten rheological properties in relation to wheat cultivar and protein composition (Khatkar et al., 1995; Janssen et al., 1996; Edwards and Dexter, 1999; Tronsmo et al., 2003). These studies showed that fundamental testing can differentiate the glutens from flours of different bread-making qualities. Furthermore, there was a significant influence of N fertiliser level on the viscoelastic characteristics in the work of Tronsmo et al. (2003).

Baking quality was evaluated using principal component analysis (PCA) of the protein and gluten contents of wheat following grass-clover and cereal, the rheological characteristics of gluten, the water absorption of the dough, and the loaf volume. In the PCA shown in figure 5 PC1 and PC2 explained 86% of the variability in the characteristics. The loading plot in Figure 5 (B) shows that PC1 mainly describes the variability in the gluten rheological characteristics (creep, recovery, G' and G"). The variability in protein, gluten, water absorption and calculated rheological characteristics (delta and % recovery) were associated with the second PC. The loading plot shows some distinction between the rheological variables, in particular between δ at 1 Hz (delta1) and δ at 0.01 Hz (delta 0.01). Protein and gluten content, and water absorption were the characteristics most strongly related to loaf volume, whereas the rheological characteristics showed weaker correlations. However, the delta1 value was close to that of loaf volume, which indicates that high protein and gluten contents are reflected in higher δ values at 1 Hz (delta1). This agreed well with results of Tronsmo et al. (2003), who also found that increasing N-fertilisation results in glutens that are more viscous and less elastic. Regarding protein composition, earlier findings demonstrate an increase in the ratio of gliadins to glutenins with increasing N-fertilisation (Daniel and Triboi, 2000; Tronsmo et al., 2003), which increases the viscous properties of the glutens (Khatkar et al., 1995).

The scores plot in Figure 5 (A) shows that samples were widely distributed in the four quadrants of the scores plot, due to the variability in rheological characteristics, protein and gluten contents, and loaf volume. Some variability was observed in samples receiving identical fertiliser treatments, in particular in samples following cereals (cer), and samples following grass-clover (clo0 and clo115). For samples from mineral fertilised cereals (cer) the variability may be explained by the low gluten content. This makes the washing of gluten more complicated, which results in higher variability in the following rheological measurements. For samples following grass clover (clo), the variability could be explained by the influence of ley age. Along the PC1 axis there was no distinction between mineral fertilised cereal and grassland precrops (cer and clo). However, along the PC2 axis samples from mineral fertilised cereals (cer) have low scores, whereas ploughed grasslands (clo) have higher scores. Increasing N-applications are reflected in higher scores for PC2, mainly for samples following cereals (cer). Scores for mineral fertilised cereals with N-applications of 125 and 150 kg N ha^{-1} (cer125 and cer150) were close to those of grassland receiving 0 and 115 kg N ha^{-1} in cattle slurry (clo0 and clo115). Therefore, the quality characteristics of wheat following an unfertilised grass-clover pre-crop corresponded to the quality obtained from the mineral fertilised wheat following cereals receiving 125-150 kg N ha^{-1}.

The PLSR of the loaf volume as y-variable showed that protein, gluten and water absorption were the major variables predicting loaf volume. With these three x-variables the validation variance of loaf volume was 74%, and the correlation coefficient between measured and predicted was r=0.81. Using only rheological characteristics of gluten as x-variables, 55% of the variation in loaf volume could be predicted with r=0.68 %. This agrees well with Tronsmo et al. (2003), who found validation variance of 58% for loaf volume and r=0.72. The most important rheological characteristics in the PLSR model were delta1 and % recovery. Including these variables together with protein, gluten and water absorption resulted in a slightly higher validation variance of 75% and r=0.83 between predicted and measured loaf volume. This small increase in validation variation and correlations using rheological characteristics and protein and gluten reflects a low covariance between protein and gluten content and the rheological parameters.

A more detailed analysis of the results from the grass-clover showed that increased protein and gluten contents due to slurry application had no effect on loaf volume following 1 and 2-year-old leys, whereas a significant increase (P<0.001) was shown after 8-year-old leys (Fig. 6). This corresponds with the results from the PLSR where protein and gluten explained only 32% of the variability in loaf volume in grass-clover samples. The same results were obtained with rheological characteristics as x-variables. However, when protein, gluten, water absorption and rheological characteristics were included, the validation variance increased to 59%. This could be further improved to 78% (r=0.74) when ley age was also included in the model. Results from this PLSR are shown in Figure 7. The loadings plot (Fig. 7B) shows that protein, gluten and age are positively correlated to loaf volume, whereas the moduli (G' and G'') are negatively correlated. These X-variables were also found to be significant in PLSR model. This indicates that besides the increase in protein and gluten contents with slurry application, a change in gluten rheological properties also takes place. This variability in rheological properties had a significant influence on loaf volume. The increase in bread volume for 8-year-old leys agreed with the previously mentioned suggestion, that N mineralisation occurred later in the 8-year leys, and this may have an influence on the protein composition of the grain.

3.4 Nitrate leaching

Rainfall in both hydrological years (April to March) from 2002 to 2004 was just over 600 mm, which is well below the annual mean of 770 mm at this site. The drainage to below the root zone (1 m) was 234 and 290 mm in 2002-3 and 2003-4, respectively, and the corresponding nitrate leaching was 20 and 13 kg N ha^{-1} (Fig. 8). Thus, the annual flow-weighted mean nitrate concentration (nitrate leaching per volume drainage) decreased from 8.5 mg N l^{-1} in year 1 after grassland cultivation to 4.5 mg N l^{-1} in year 2. Nitrate leaching in year 1 after ploughing was significantly influenced by type of grassland ($P<0.001$) with 10 and 29 kg N ha^{-1} leached from grass-clover and ryegrass, respectively.

Nitrate leaching following ploughing of 1-year-old leys averaged 11 kg N ha^{-1}, which was significantly lower than the 24 kg N ha^{-1} following 2 or 8-year-old leys. This pattern was also evident in year 2 following ploughing with values of 9 and 16 kg N ha^{-1} leached following 1-year-old ley and 2 or 8-year-old leys, respectively. Although there was a tendency for fertiliser application to increase leaching, this was not significant, which may seem surprising considering the lack of yield response to fertiliser application. However, in year 2 there was a clear effect of fertiliser ($P<0.001$), which may, at least partly, be caused by the cumulative effect of annual fertiliser application to the same plots. Considering that grassland cultivation is the potentially most leaky place in the crop rotation, nitrate losses were low in this study. This was probably only possible because of an efficient catch crop (Sherpherd and Lord, 1996).

It was striking that the EU Drinking Water Directive upper limit of 50 mg nitrate per litre was on average not exceeded in year 1 after grassland cultivation in any of the plots where grass-clover was ploughed out, but was exceeded in all plots where ryegrass had been grazed for more than 1 year (Fig. 8). In the 2nd winter (2003-4) the 50 mg nitrate per litre was on average not exceeded in any instance (Fig. 8), although nitrate leaching following grazed ryegrass was higher than following grazed grass-clover.

3.5. General discussion

The huge residual effect of grasslands compared with the reference area previously cropped with cereals indicates a substantial N accumulation in the ley fields, of which a large proportion was released in the first year after ploughing. Others have found that the residual N effect of grassland decreased rapidly during the three years after ploughing up the grassland (Nevens and Reheul, 2002; Eriksen, 2001). However, the residual effect was also considerable in the second year following grassland ploughing, probably caused by a combination of efficient catch crops and low winter rainfall between the years.

The N-release from grasslands considerably increased yields and - to en even greater extent - the baking quality of the spring wheat. With grass-clover residual effect as the sole source of N, the wheat flour contained 12.0% protein and 23.5% gluten, which are values that confer good bread-making qualities. Application of N in slurry increased the protein, gluten and total protein accumulation. However, the application of slurry had almost no effect on the loaf volume. The quality of the spring wheat supplied with mineral fertiliser varied widely from 9.5 to 12.8% protein and 12.4 to 27.4% gluten. Protein and gluten in wheat following cereals with 125 kg mineral N ha^{-1} application corresponded to the values following grass-clover with no addition of N (12.0% protein and 23.5% gluten). The grain yield for 125 kg mineral N ha^{-1} was slightly higher compared with the grass-clover with no fertiliser application. However, the rheological properties of gluten from these two treatments differed considerably. Gluten in wheat following grass-clover had a higher extensibility and recovery measured by creep recovery and was more viscous (lower G' and higher δ), and the loaf volume at 420 mL was slightly higher than the 390 mL following cereals with 125 kg mineral N ha^{-1}. The results from this experiment indicate that the gluten properties and the loaf volume seem to be affected by the N source.

The quantity of N accumulated during the ley phase of the cropping system depends on the equilibrium between inputs and the soil organic N pool, which in turn depends on the age of the ley and the botanical composition. In a system without grazing, Johnston et al. (1994) found that N accumulation reached a maximum in third-year grass-clover. When maximum accumulation has been reached, excess N inputs are likely to be lost. Thus, Ledgard et al. (1999) found the measured N surplus of permanent pastures to be equal to the combined losses by nitrate leaching, ammonia volatilisation and denitrification. Similarly, Scholefield et al. (1993) found that nitrate leaching losses were 50% lower in reseeded pasture compared with permanent pasture. In this study the accumulated differences in N surplus were considerable for the different types of leys and for different ages. Eriksen et al. (2004) estimated the N surplus (fixation/mineral N input + animal excreta – herbage output) of the grass-clover and grass leys to be c. 50 and 240 kg N ha^{-1} yr^{-1}, respectively, in production years 5-8. The lower accumulation in grass-clover is explained by a reduction in N_2-fixation in grass-clover over time and a reduction in dry matter production in grass-clover over time, lowering the grazing intensity and the recycling of grassland N via animal excreta. Under similar conditions and for the same soil type Hansen et al. (2005) estimated the N surplus of 1^{st} and 2^{nd} year grazed grass-clover to be 122 and 245 kg N ha^{-1} yr^{-1}. On the basis of the expected, large differences in N surplus and N accumulation in the different grasslands, larger differences in N-release might have been expected. However, the total N output in crop uptake and nitrate leaching (Fig. 9) was only marginally affected by these differences. In the two years following ploughing of grass-clover the N output in unfertilised plots was 250-260 kg N ha^{-1} independent of grassland age and for the grass only ley the total output was 271-306 kg N ha^{-1}, increasing with age. Similar results have been found by Hansen et al. (2005), who suggested that the inconsistency between accumulated N and residual effect indicates that organic N is more easily mineralised the more recently it has been formed. The present results furthermore suggest that the N accumulated in older grasslands is released more slowly following ploughing. This was indicated by the plant N partitioning (see above) and the larger proportion of N output being in the form of nitrate leaching following the ploughing of older grasslands compared with short-term grass leys.

4. Conclusion

More N was released following ploughing of ryegrass swards due to more intensive use (Eriksen et al., 2004) and more N was also released from grassland of increasing age as evidenced by plant residue N at incorporation of the swards, N fertiliser replacement value following incorporation and nitrate leaching the following winter. However, these differences were moderate compared to the considerable differences in N surplus that may be estimated for grasslands of different age and use. Thus, the results indicate that when accumulated organic matter is partially decomposed and the readily mineralisable N used, the remaining organic N is released only very slowly. This will in turn lead to more nitrate leaching even when good management is practised as in this experiment (spring ploughing, catch crops and reduced fertiliser input). The low nitrate leaching following ploughing of the less intensively used grass-clover swards indicates that the self-regulatory nature of the grass-legume association offers an opportunity for increasing nutrient use efficiencies in mixed farming systems.

Irrespective of composition, the leys gave rise to production of good quality bread wheat especially following longer-term swards. Besides satisfactory contents of protein and gluten, the rheological properties of the gluten following grass-clover were different from gluten achieved using equivalent quantities of mineral fertiliser. The variability in rheological properties between mineral fertilised wheat and grass-clover wheat indicates that the farming system has a considerably effect on the functional quality of the wheat and on the loaf quality. The slow release of N from decomposition of old grassland probably gave a better synchrony between N release and plant demand.

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Tables

Table 1. Incorporated plant residues (tops and roots to 20 cm)

Plant residue	Prior use in 2001	DM ($t\ ha^{-1}$)	CV (%)	Root-top ratio	N ($kg\ ha^{-1}$)	C/N
Pasture 2002	Grass-clover; 1 yr	6.6	28	12.2	144	19
	Grass-clover; 2 yr	7.9	20	15.8	161	20
	Grass-clover; 8 yr	10.3	19	20.1	200	22
	Ryegrass; 1 yr	8.2	23	9.4	176	19
	Ryegrass; 2 yr	8.3	12	8.7	194	18
	Ryegrass; 8 yr	9.0	12	11.5	213	18
	Cereals	-	-	-	-	-
Catch crop 2003	Grass-clover; 1 yr	3.8	33	2.9	73	22
	Grass-clover; 2 yr	3.2	24	2.9	64	22
	Grass-clover; 8 yr	4.2	47	2.4	78	23
	Ryegrass; 1 yr	3.3	33	2.5	66	22
	Ryegrass; 2 yr	5.8	46	5.0	122	20
	Ryegrass; 8 yr	3.4	23	2.0	66	22
	Cereals	4.7	17	4.2	99	20

Legends

Fig. 1. Design of field experiment. Numbers 1, 2 and 8 refer to the age of grass leys at the time of ploughing.

Fig. 2. Yield and N uptake in grain and straw of spring wheat following unfertilised cereals and grass leys of different age. Error bars: SE.

Fig. 3. Yields of wheat grain and barley wholecrop in the two years following ploughing of grasslands and compared to similar yields following a cereal history. Error bars: ±SE.

Fig. 4. Protein, starch and gluten contents of wheat grain following ploughing of grasslands and compared to similar yields following a cereal history. Error bars: ±SE.

Fig. 5. PCA scores (A), and loadings (B) for quality parameters of spring wheat following mineral fertilised cereals (cer with 0, 25, 50, 75, 100 and 125 kg N ha^{-1}) and following grass-clover (clo with 0, 115 and 230 kg total-N ha^{-1}).

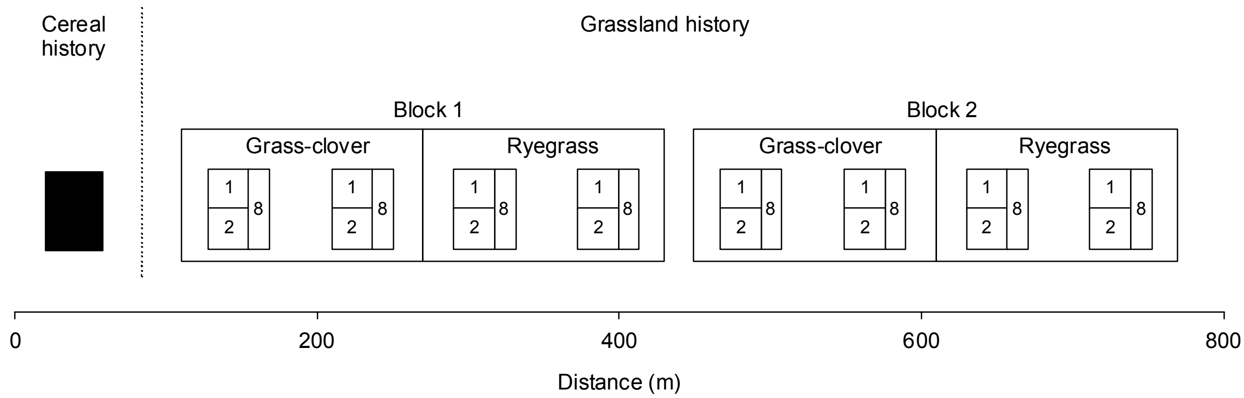
Fig. 6. Protein and gluten contents of flour, and loaf volume at different N application rates (0, 115 and 230 kg total-N ha^{-1}) and different ages of grass leys (1, 2 and 8 years).

Fig. 7. PLSR scores (A) and loadings (B) for volume (y-variable) and rheological parameters, protein, gluten and water absorption (x-variables) of spring wheat following grass-clover (clo with 0, 115 and 230 kg total-N ha^{-1})

Fig. 8. Nitrate leaching in cereals in the two years following ploughing of six grasslands. The dotted line indicates nitrate leaching corresponding to the EU Drinking Water Directive upper limit of 50 mg nitrate l^{-1}.

Fig. 9. The accumulated N output in nitrate leaching and crop removal in the two years following ploughing of six grasslands. Error bars: SE.

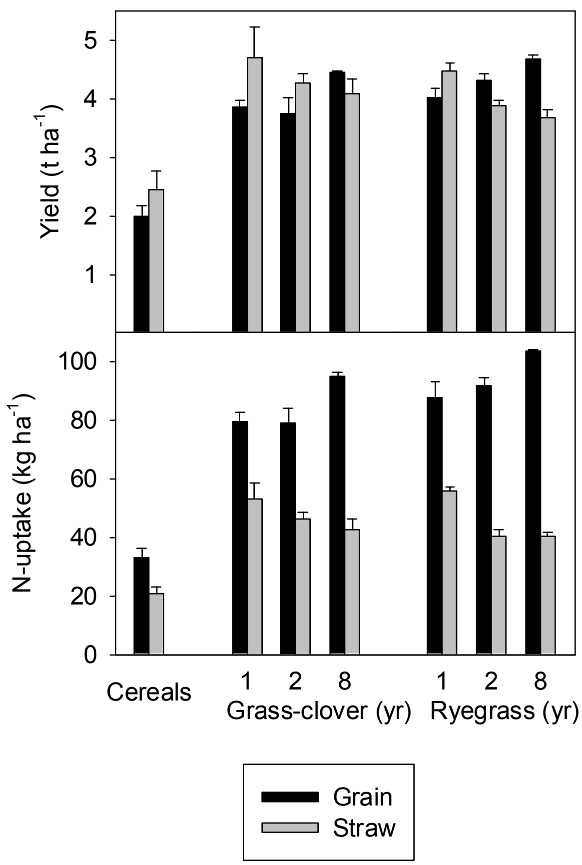
Figure



Cereal history
Grassland history
Block 1
Block 2
Grass-clover
Ryegrass
Grass-clover
Ryegrass
1
2
8
0
200
400
600
800
Distance (m)

Fig. 1

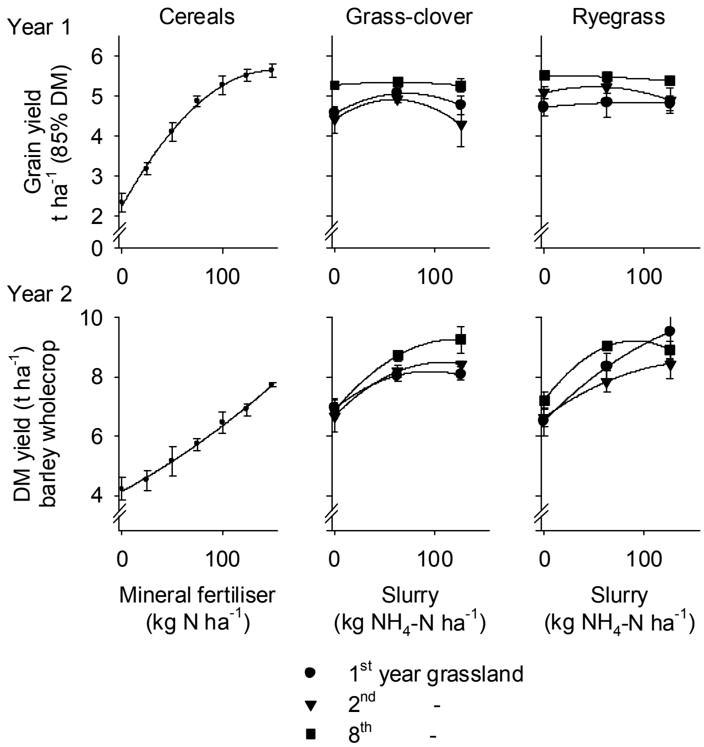
Figure



5
4
3
2
1
Yield (t ha^{-1})
100
80
60
40
20
0
N-uptake (kg ha^{-1})
Cereals
1
2
8
Grass-clover (yr)
1
2
8
Ryegrass (yr)
Grain
Straw

Fig. 2

Figure



Year 1
Cereals
Grass-clover
Ryegrass
Grain yield
t ha-1 (85% DM)
Year 2
DM yield (t ha-1)
barley wholecrop
Mineral fertiliser
(kg N ha-1)
Slurry
(kg NH4-N ha-1)
Slurry
(kg NH4-N ha-1)
1st year grassland
2nd -
8th -

Fig. 3

Figure

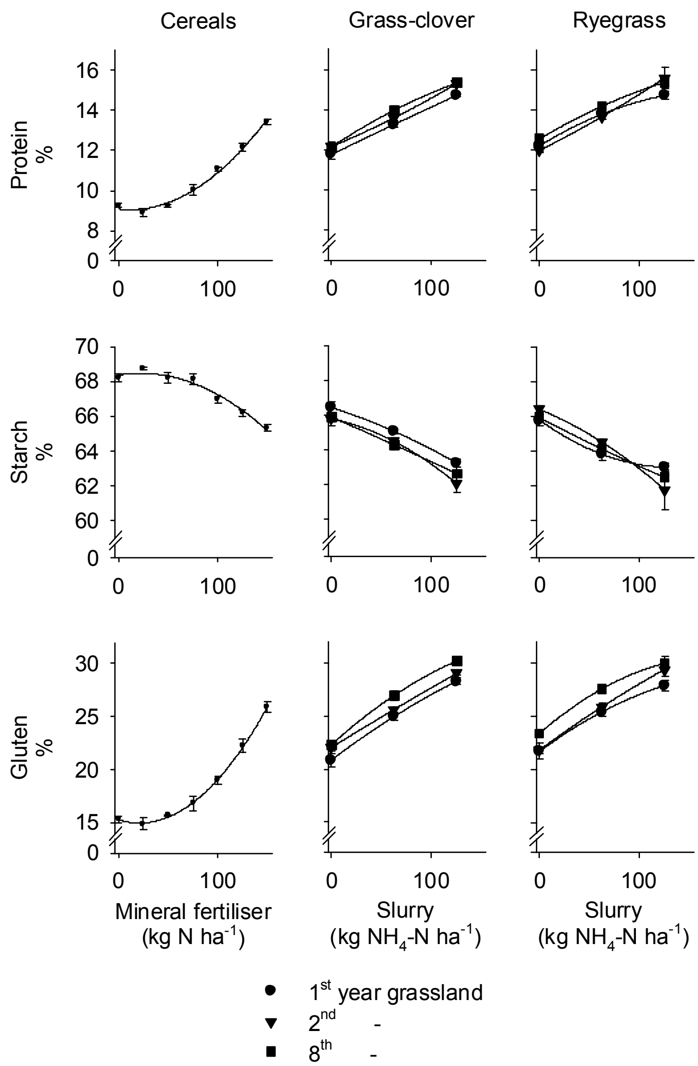

Cereals
Grass-clover
Ryegrass
Protein %
Starch %
Gluten %
Mineral fertiliser (kg N ha-1)
Slurry (kg NH4-N ha-1)
Slurry (kg NH4-N ha-1)
1st year grassland
2nd -
8th -

Fig. 4

Figure

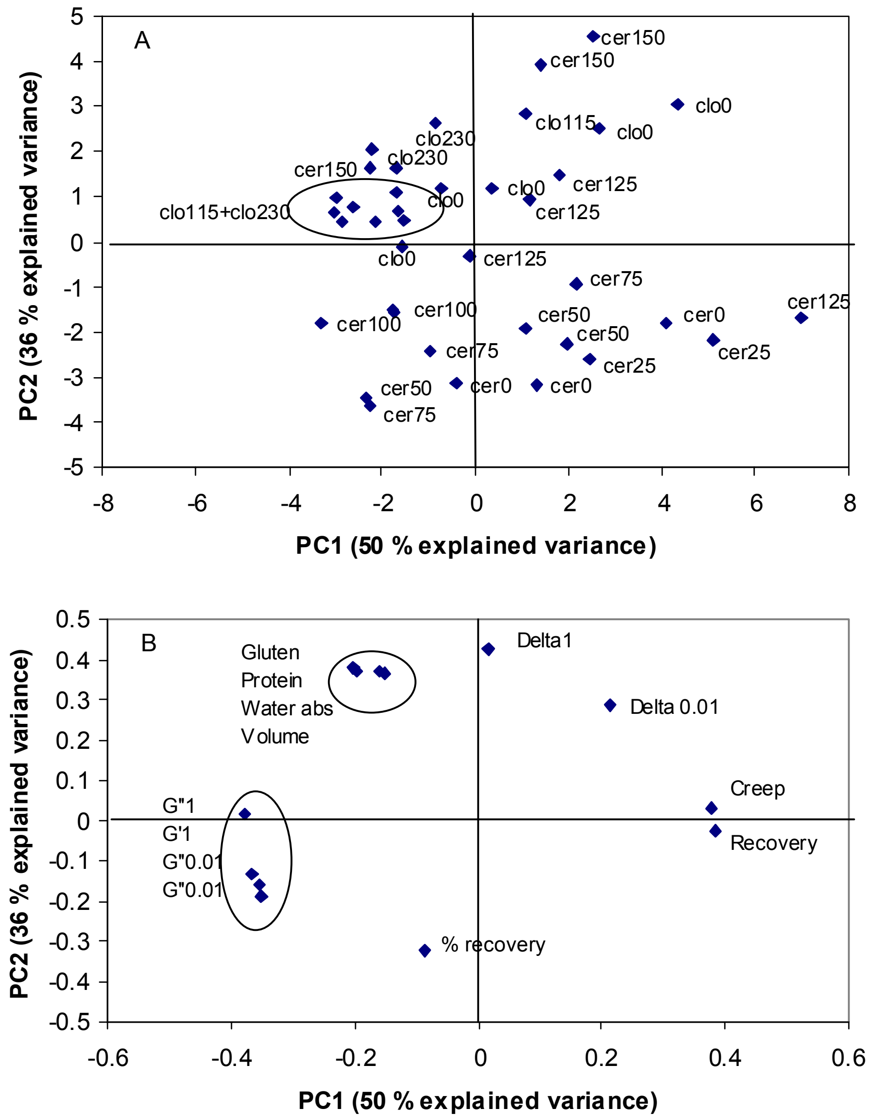
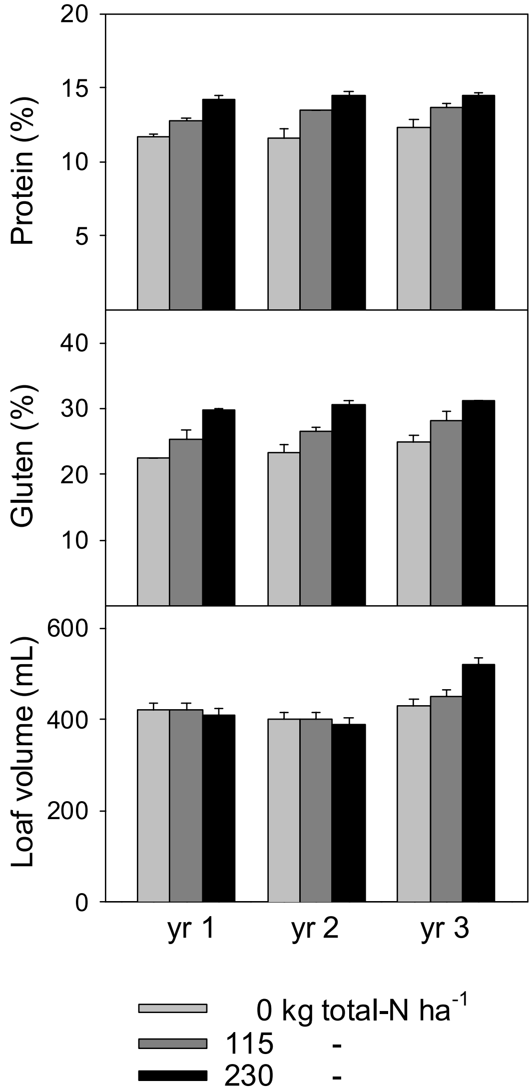

A
cer150
cer150
clo0
clo115
clo0
clo230
clo230
cer150
clo0
cer125
clo115+clo230
clo0
cer125
clo0
cer125
cer75
cer100
cer100
cer50
cer0
cer125
cer50
cer25
cer75
cer25
cer50
cer0
cer0
cer75
PC2 (36 % explained variance)
PC1 (50 % explained variance)
B
Gluten
Protein
Water abs
Volume
Delta1
Delta 0.01
Creep
Recovery
G"1
G'1
G"0.01
G"0.01
% recovery
PC2 (36 % explained variance)
PC1 (50 % explained variance)

Fig. 5

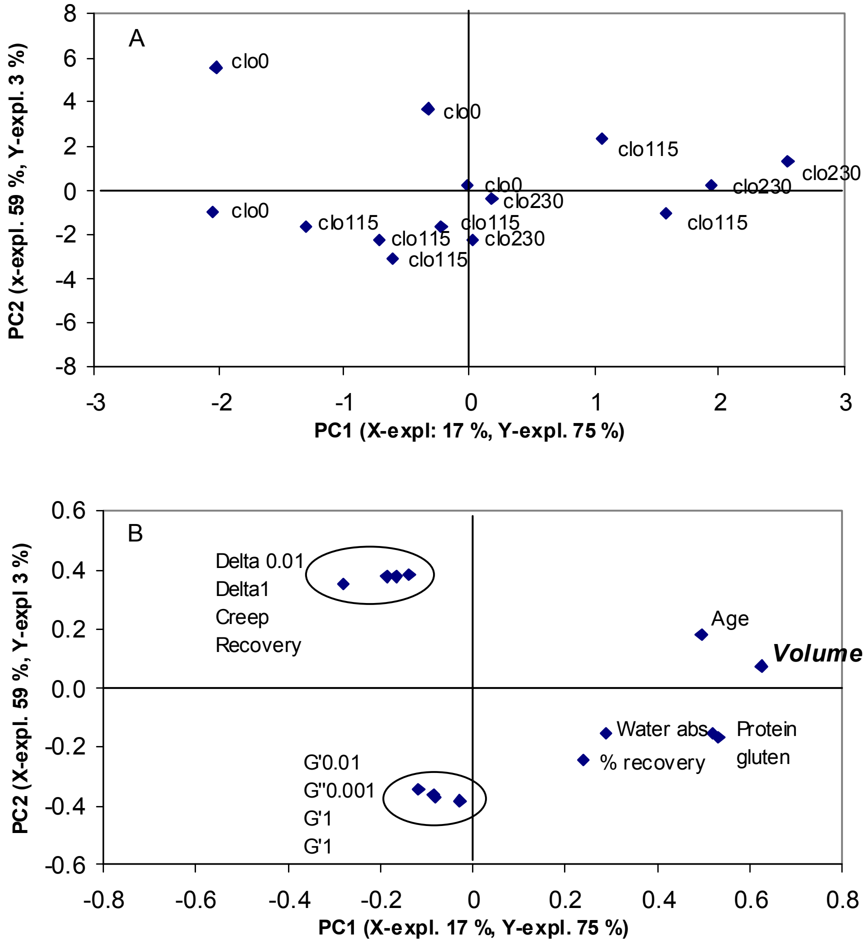
Figure



20
15
10
5
Protein (%)
40
30
20
10
Gluten (%)
600
400
200
0
Loaf volume (mL)
yr 1
yr 2
yr 3
0 kg total-N ha-1
115 -
230 -

Fig. 6

Figure



A
clo0
clo0
clo115
clo230
clo0
clo230
clo230
clo0
clo115
clo115
clo115
clo230
clo115
clo115
PC2 (x-expl. 59 %, Y-expl. 3 %)
PC1 (X-expl: 17 %, Y-expl. 75 %)
B
Delta 0.01
Delta1
Creep
Recovery
Age
Volume
Water abs
Protein
% recovery
gluten
G'0.01
G"0.001
G'1
G'1
PC2 (X-expl. 59 %, Y-expl 3 %)
PC1 (X-expl. 17 %, Y-expl. 75 %)

Fig. 7

Figure

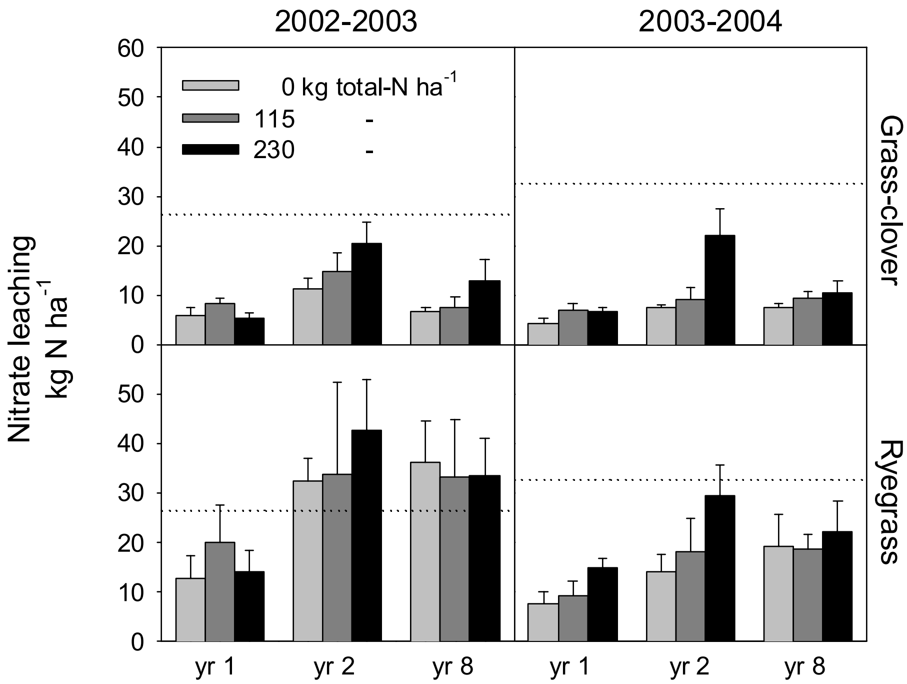
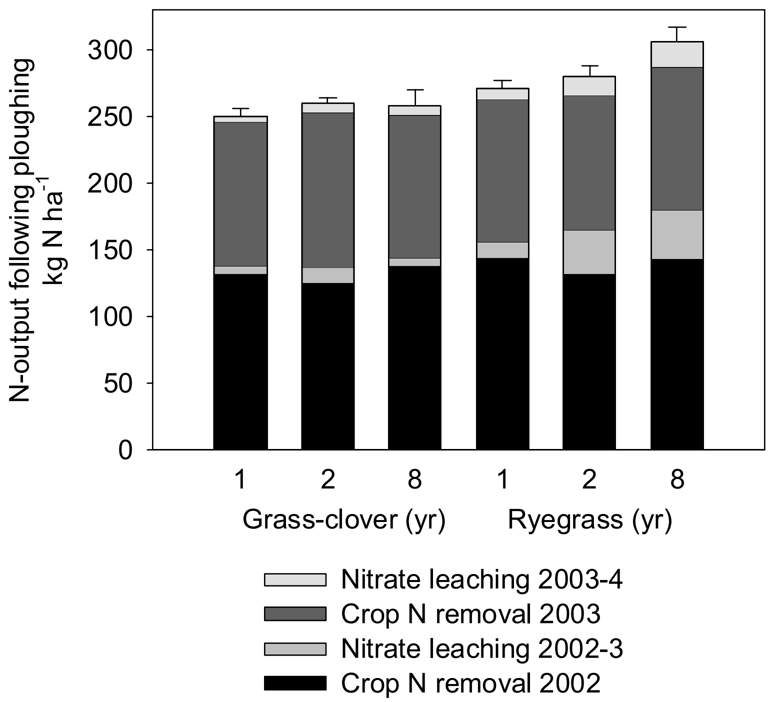

2002-2003
2003-2004
0 kg total-N ha-1
115 -
230 -
Nitrate leaching
kg N ha-1
60
50
40
30
20
10
0
Grass-clover
Ryegrass
yr 1
yr 2
yr 8
yr 1
yr 2
yr 8

Fig. 8

Figure



300
250
200
150
100
50
0
N-output following ploughing
kg N ha-1
1
2
8
1
2
8
Grass-clover (yr)
Ryegrass (yr)
Nitrate leaching 2003-4
Crop N removal 2003
Nitrate leaching 2002-3
Crop N removal 2002

Fig. 9