Report to the Stapledon Memorial Trust

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RESEARCH ON NUTRIENTS IN CUT GRASSLAND RECEIVING LONG-TERM MANURE APPLICATIONS

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Introduction

The main purpose of the Fellowship

The application for this fellowship followed the attendance by Professor Yongguan Zhu at the International Grassland Congress held in Dublin in July 2005. The visit to Dublin re-established a previous link between Professor Zhu and Dr Peter Christie of Queen's University Belfast and the Department of Agriculture for Northern Ireland (DARD). During the Congress both Dr Christie and Professor Zhu felt that a DARD long-term slurry experiment would provide an excellent opportunity to investigate the influence of soil nutrient management on grassland diversity and productivity, particularly from the point view of ecological stoichiometry following long-term inputs of different nutrient sources and levels.

Recycling of animal manures is a key to the maintenance of environmental quality while maintaining optimum productivity of livestock systems. In order to predict the impacts of the application of animal manures on grassland productivity and soil quality, a long-term field experiment was established in Northern Ireland in 1970. After 35 years, different treatments have produced significant differences in cut herbage production and sward botanical composition as well as soil fertility. In recent years, ecological stoichiometry has been proposed to study the response of plant communities to changes in soil fertility and other environmental conditions (Elser et al., 2000). It is thought that the changes in soil fertility and plant stoichiometry in consequence may influence plant diversity (Guswell et al., 2005). However, most studies so far have focused only on spatial variation in plant N:P:C ratios or on short-term plot and pot experiments. The DARD long-term field experiment provides a rare opportunity to examine the relationship between soil fertility and grassland biodiversity together with the practical dimension of recycling of animal manures.

The working hypothesis of the study is that emerging plant species in low input grassland may have a distinct signature of nutrient composition, particularly N and P (i.e. ecological stoichiometry), and that their nutrient stoichiometry may provide a useful tool for predicting species dynamics in grassland under various conditions of soil fertility. This study builds on a successful long-term study on soil fertility and grassland productivity, and the proposed research will be a value-added investigation since the topic of the study is prompted by some recent research developments in ecological and grassland studies. We would hope that the results will provide novel information on the influence of soil nutrient management on grassland diversity.

The Fellowship has also contributed to my training in an international context and in grassland systems in particular, and has helped to improve my scientific communication skills in English. I also benefited immensely from the cultural aspects of my visit to the UK.The Fellowship started in early April and ended at the end of September in accordance with the main grass growing season.

The research project

General introduction
My practical work comprised two main parts. The first was to separate herbage plant species from each of 48 plots in a field experiment to determine the botanical composition and plant biodiversity under the different long-term treatments. I did this part of the work with Dr Scott Laidlaw and his staff at the DARD Plant Testing Station at Crossnacreevy near Belfast. Dr Laidlaw trained me in the identification of the plant species in the herbage samples. The second part of the work was the chemical analysis of the separated plant samples. This was done in cooperation with Dr Peter Christie and his research assistant Elizabeth Anne Wasson in the Agricultural and Environmental Science Department at Queen's University Belfast. All of the staff at both centres was very helpful to me. I have collected a large amount of data on botanical composition, herbage species diversity and chemical analysis of nutrient elements at the first silage cut and also some data on botanical composition and plant diversity at the second cut for initial interpretation in this report.
In addition, I was also involved with Dr Laidlaw's research group in some on-farm experiments about white clover on organic farms and this gave me further opportunity to visit numerous farms around Northern Ireland. I really appreciate Scott's help and the opportunity this gave me to see numerous parts of the Province.

The long-term slurry experiment

My research project was based on a long-term field experiment that was established on a sown sward of perennial ryegrass (*Lolium perenne*) at Hillsborough, Northern Ireland, in 1970. There were eight treatments: unfertilized control (UFCTRL), fertilized control (FECTRL: 200 kg N, 32 kg P, and 160 kg K $ha^{-1}y^{-1}$), pig slurry at 50, 100 and 200 m^3 $ha^{-1}y^{-1}$ (Pig50, Pig100 and Pig200) and cow slurry at the same three rates of slurry (Cow50, Cow100, Cow200). There were six replicates giving a total of 48 plots in a randomized-block design. The sward was cut three times each year at the silage stage (17 May, 18 July and 19 September 2006). As I did not have enough time to separate the individual grass species and perform all of the chemical analysis on herbage from the third cut during my visit from April to October, the samples have been retained in a freezer at the Plant Testing Station for local staff to complete the work during the 2006-2007 winter. The fertilizers and slurries were applied in three equal dressings, first in the early spring and then immediately after the first two cuts. Each rectangular plot is 29.75 m^2 in area and herbage was cut with a plot harvester and weighed in the field. Two kinds of herbage samples were collected at three cuts. Routine samples were taken and then oven dried at 80°C for dry matter determination and ground to pass a 0.5-mm sieve prior to chemical analysis. Additional samples for species separation were taken at the same time and stored at -20°C for subsequent determination of the botanical composition and stoichiometry of nutrient elements in the dominant plant species.

Sward botanical composition

Herbage samples, at least 1 kg fresh weight, for botanical separation were collected from each plot at all three cuts in 2006. These were separated as far as possible into individual plant species which were then oven dried at 60°C for 24 hours and their contribution to herbage dry matter calculated. Dominant species for each treatment were then determined. Replicates within each treatment were paired (Table1) and the two samples of each dominant species bulked so that there were three replicates per treatment of each dominant species. From the botanical analyses data, three main species were identified i.e. *Lolium perenne* L. (perennial ryegrass), *Agrostis stolonifera* L. (creeping bent), and *Poa* spp., except for the treatment without fertilizer (UFCTRL) in which the main species were *Lolium perenne* L., *Agrostis tenuis* (common bent) and *Holcus lanatus* (Yorkshire fog).

Chemical analysis in dominant species of herbage

The separated samples of the dominant herbage species were oven dried at the same temperature as the routine samples for 48 hours prior to chemical analysis and then ground to pass a 0.2-mm sieve in an Ultra Centrifugal Mill ZM200 (Retsch Company, Germany). The total nitrogen and carbon in plants were measured with LECO 2000 Dry Combustion Analyzer (LECO Equipment Corp., St. Joseph, MI). The other elements were detected using an Axios X-Ray Fluorescence spectrometer (XRF) (PANalytical B. V., the Netherlands), including phosphorus (P), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn). Pairs of replicate samples were combined before chemical analysis (Table 1).

Table 1 Pairing of replicates for bulking dominant species

	Treatments							
	FE CTRL	UF CTRL	Pig 50	Pig100	Pig200	Cow50	Cow100	Cow200
Replicate1	4, 9*	5,11	8, 10	3,14	6, 16	2, 13	1, 7	12, 15
Replicate2	29, 31	27, 30	17, 22	25, 28	24, 26	18, 21	19, 23	20, 32
Replicate3	33, 48	43, 45	39, 47	34, 41	35, 42	38, 44	36, 37	40,46

* No. of each plot

Statistical analysis

Diversity indices and evenness were calculated using the Shannon-Wiener equations. Analysis of variance (ANOVA) on the yield of herbage, biodiversity indices, and the N: P ratio was performed using SPSS Version10.0. Tukey's HSD multiple comparison tests were carried out.

Results

Herbage dry matter yield

Dry matter yields of herbage from the first cut are presented in Figure 1. The data show that sward yields were lowest without fertilizer (UFCTRL), a similar effect to that found in 1981 (Christie, 1987). The yields in other treatments were significantly higher than UFCTRL ($P<0.001$). The yields in other treatments were significantly higher than UFCTRL ($P<0.001$). The general trends are similar to those for yields at the second cut and no significant difference was found between the two cuts (data from cut 2 not shown here). At Cut 1 the dry matter yield markedly increased with increasing rate of application of pig slurry. Yield of Pig200, Cow100 and Cow200 did not differ significantly. At rates lower than 200 m^3 ha^{-1} cow slurry treatments were significantly higher than the corresponding pig slurry treatment. (Figure1). This may have been due to the significantly lower total nitrogen, ammonium-nitrogen, phosphorus and potassium contents in pig slurry than in cow slurry at the first application (total N%, NH_4^+-N%, P%, K%: cow slurry 0.39, 0.24, 0.08 and 0.93; pig slurry 0.08, 0.06, 0.02 and 0.11). The herbage yield of Cow50 was significantly lower than that of Cow100 or Cow200.

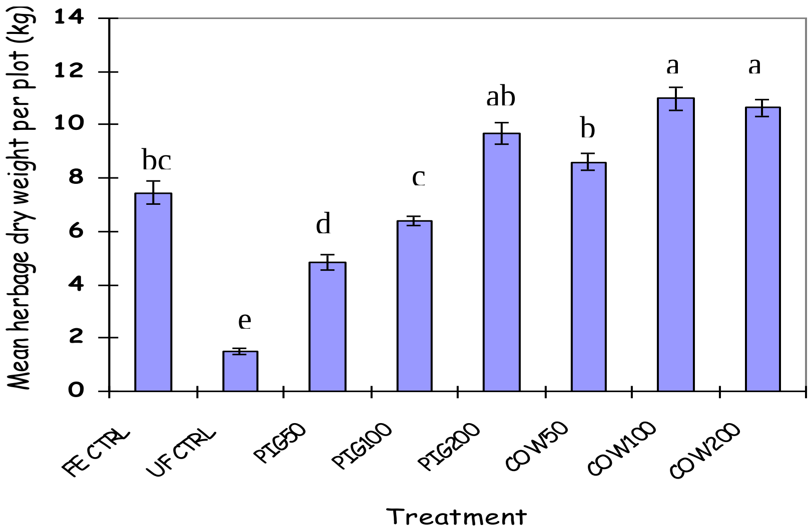

Figure1 Total herbage dry matter yields in different treatments (mean±SE, n=6). Different letters indicate the results of multiple comparisons according to Turkey's HSD test

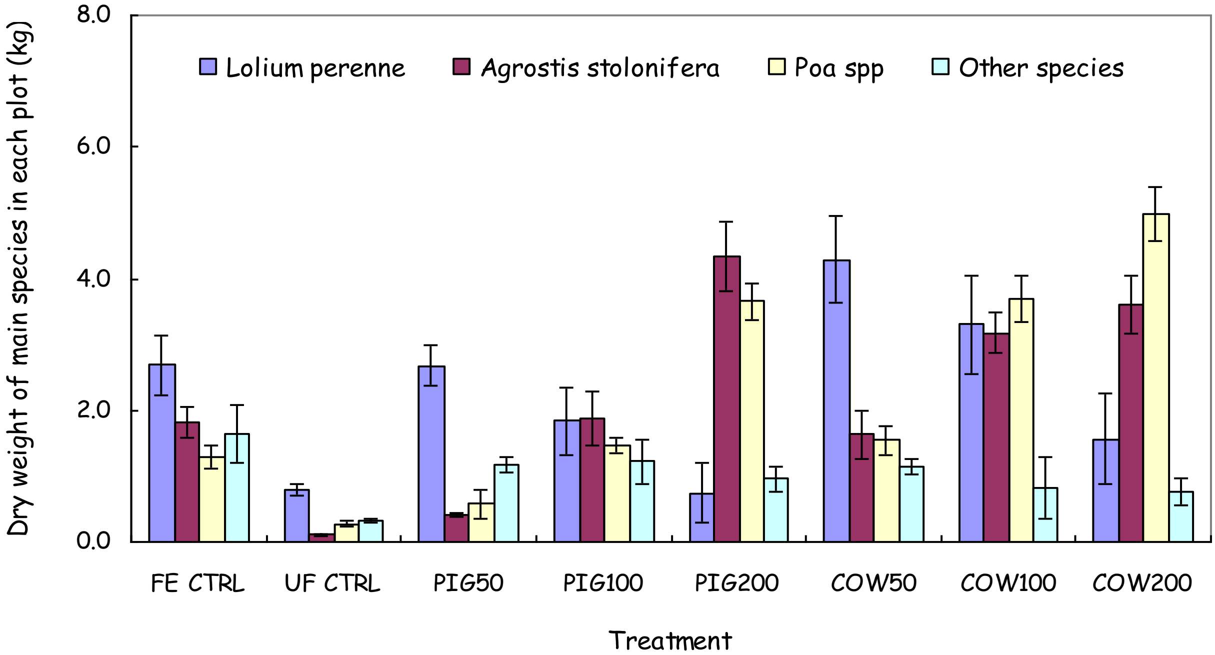

Figure 2 Distribution of dry matter in main herbage species (mean±SE) n=3. In UFCTRL the main species were *Agrostis tenuis* and *Holcus lanatus*

However, *Lolium perenne* L., one of the dominant species, had the highest yield in the cow50 treatment and was significantly higher than in other treatments including UFCTRL (P<0.001)

(Figure 2) (botanical composition will be described later). Thus, the yield of *Lolium perenne* decreased markedly with increasing rate of slurry application. Cow200 and Pig200 had lower yields of *Lolium perenne* and similar to UFCTRL. In contrast, the yields of *Agrostis stoloniferra* and *Poa* spp., the main invading species, increased with increasing application rate of both pig and cow slurries (Figure 2). *Agrostis stolonifera* had the highest yield in Pig200 and *Poa* spp. had the highest yield in Cow200. Therefore, different application rates of pig and cow slurries changed the botanical composition and the distribution of the main grass species

Sward botanical composition and plant diversity index in the long-term slurry experiment
The botanical composition changed markedly in the proportions of the sown grass species (*Lolium perenne*) and unsown species in different treatments after 35 years (data not shown). The main invading species were *Agrostis stolonifera* and *Poa* spp. in all treatments except for UFCTRL in which they were *Agrostis tenuis* and *Holcus lanatus*. Other species which ingressed, but in lower proportions or traces, were *Dactylis glomerata, Phleum pratense, Agropyron repens, Festuca rubra, Alopecurus pratensis, Taraxacum officinale, Anthoxanthum odoratum, Ranunculus acris, Trifolium repens, Cerastium arvense,* and *Bellis perennis.* Botanical composition of the various treatments were quite similar to that reported in 1981-1982 (Christie, 1987) except for UFCTRL treatment in which the main grass species were L*olium perenne, Agrostis tenuis and Holcus lanatus*. Moreover, there were also some large proportions of other unsown grasses in UFCTRL, for example *Festuca rubra, Anthoxanthum odoratum and Trifolium repens.*

The high contribution of *Agrostis stolonifera* and *Poa* spp. in the treatments with medium and high application rates of pig and cow slurries is interesting. Although large enough amounts of *Agostis stolonifera* and *Poa* spp. from the whole samples were separated to conduct chemical analysis on samples from most of the treatments, enough *Agrostis stolonifera* or *Poa* spp. could not be collected from UFCRTL; instead, *Agrostis tenuis* and *Holcus lanatus* were separated and analysed chemically, being the dominant invading species in the no-fertilizer control.

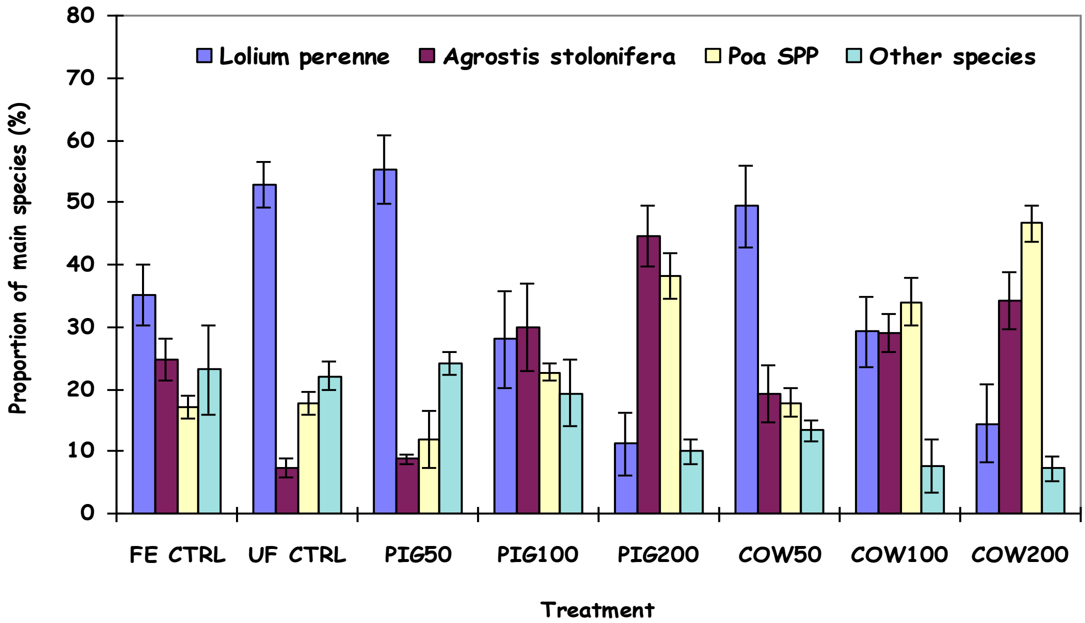

Figure 3 The percentages of the main species in dry matter yield of herbage, the main species being *Agrostis tenuis* and *Holcus lanatus* in UFCTRL

The proportions of the three main species of the herbage (*Lolium perenne*, *Agrostis stolonifera*, and *Poa* spp.) are shown in Figure3. *Lolium perenne* is one of the dominant species in each plot for treatments FECTRL, UFCTRL, Pig50 and Cow50. The proportion of *Lolium perenne* in treatments UFCTRL, Pig50 and Cow50 exceeded 50% of total dry matter yield. However, *Agrostis stolonifera* and *Poa* spp. were two invading dominant grass species in Pig200 and Cow200. There were no significant differences between the proportions of *Lolium perenne, Agrostis stolonifera,* and *Poa* spp. in Pig100 and Cow100. Thus the distribution of the three grass species were even in each plot of the medium levels of slurry application if remaining mixture of other species was collectively regarded as a component of the main species (Figure3). Differences in evenness and the proportions of the main species require further interpretation.

A further observation was that more plant species seemed to invade the UFCTRL treatment and the lowest slurry application rates than in the higher rates of cow and pig slurry *in situ*. Hence it was hypothesized that there would be higher biodiversity of plant communities in low input grassland. Therefore, plant species richness and evenness and Shannon-Wiener indices were calculated.

Species richness is the single most important component of species diversity. The evenness of species relative abundances is another key component (Krebs 1999). Recently, species richness has been largely used as the only measurement of species diversity in many studies (Ricklefs and Schluter 1993; Naeem et al., 1994; Tilman 1996). If this is so, then it is not necessary to determine species evenness and to calculate diversity index. However, when the pattern of species diversity in communities is described simply by the number of species, important aspects of the quantitative structure of communities can be missed e.g. relative abundance.

Table 2 Species diversity (Shannon index), species richness and species evenness in different treatments at the first and second cuts. Different letters in columns indicate significant differences between treatment means

	Species diversity*		Species richness		Species evenness	
Treatment	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2
FE control	1.56 a	1.55 ab	15 a	14 a	0.777 a	0.665 ab
UF control	1.42 ab	1.28 b	17 a	13 a	0.582 b	0.527 b
pig50	1.43 ab	1.86 a	17 a	14 a	0.609 b	0.751 a
pig100	1.453 a	1.18 bc	14 a	12 a	0.654 b	0.573 b
pig200	1.106 c	0.97 c	9 b	6 b	0.670 b	0.558 b
cow50	1.336 b	1.70 a	15 a	11 a	0.659 b	0.763 a
cow100	1.23 bc	1.22 b	9 b	10 a	0.769 a	0.643 ab
cow200	1.11c	1.22 b	8 b	6 b	0.684 ab	0.797 a

* Shannon index

Table 2 shows the species diversity (Shannon index), species richness and species evenness in different treatments at the first and second cuts. At the first cut (17 May), diversity index was highest in FECTRL, but there were no significant differences between FECTRL and UFCTRL, Pig50 or Pig100. Species richness was significantly higher in FECTRL, UFCTRL, Pig50, Pig100 and Cow50 than in Pig200, Cow100 or Cow200. The results also show that there was higher evenness in treatments FECTRL, Cow100 and Cow200. The trends in plant diversity at cut 2 were similar to those at cut 1. The data indicate that high rates of slurry application might decrease plant diversity (low diversity index and low species richness) in grassland after slurries have been applied routinely over many years (Table2).

The stoichiometry of nutrient elements in the long-term slurry experiment

In recent years the hypothesis has been developed that changes in soil fertility and plant nutrient stoichiometry will influence plant diversity. Therefore stoichiometry may be a useful tool for predicting plant species dynamics in grasslands with diverse soil fertility, especially for the stoichiometry of N:P ratio (on the basis of biomass).

Table 3 shows the N:P ratio in the main species under different treatments. The highest N:P ratios in the three main species occurred in the treatment without fertilizer (*Lolium perenne*: 6.57; *Agrostis tenuis*: 10.92 and *Holcus lanatus*: 8.41). N:P ratios of unsown species *Agrostis tenuis* and *Holcus lanatus* were significantly higher than that of *Lolium perenne*. Among all the treatments there were no substantial differences in N:P ratio for *Lolium perenne*, *Agrostis stolonifera* and *Poa* spp. excluding UFCTRL. This may suggest that *Agrostis tenuis* and *Holcus lanatus* invading plots without input of fertilizer had special N:P ratios and had distinct signatures of nutrient composition to indicate stronger abilities to survive in nutrient-poor soil. This result also indicates that nutrient input to grassland did not markedly change N:P ratios of *Lolium perenne, Agrostis stolonifera* or *Poa* spp.

Table 3 N:P ratio in three main species under different treatments at Cut 1. Different lowercase letters in rows indicate significant differences between main species, and capital letters in columns indicate differences among the 8 treatments. Data expressed as mean ± SE (n = 3)

Treatments	Lolium perenne	Agrostis stolonifera	Poa spp	Other species
FE CTRL	5.87±0.33 bA	7.93±0.07aB	6.10±0.11 bB	7.56±0.25 aA
UF CTRL	**6.57**±0.88 bA	**10.92**±0.53 aA*	**8.41**±0.64 abA*	**7.59**±0.74 bA
PIG50	5.01±0.07 cA	7.74±0.15 aB	5.87±0.09 bB	6.40±0.21 bA
PIG100	5.19±0.21 bA	7.35±0.09 aB	5.73±0.08 bB	6.70±0.22 bA
PIG200	4.84±0.06 cA	6.93±0.14 aB	5.56±0.27 bcB	6.17±0.26 abB
COW50	5.78±0.21bA	7.97±0.16 aB	6.10±0.13 bB	7.13±0.31 aA
COW100	6.06±0.05 bA	7.77±0.10 aB	6.31±0.30 bB	7.64±0.20 aA
COW200	6.33±0.41 cA	7.70±0.23 abB	6.64±0.05 bcB	8.06±0.13 aA

* The main species were *Agrostis tenuis* and *Holcus lanatus* in UFCTRL

It was originally hypothesised that there might be a special relationship between species diversity and N: P ratio of dominant species in different treatments. Numerous studies have focused on a possible relationship between N: P ratio in vegetation and species richness (plant diversity) using short-term pot experiments. Results suggest that there was N-limitation in soil if N: P ratio (biomass) was very low, or should be a condition with P-limitation. Addition of growth limiting nutrient elements will change the N:P ratio and increase plant diversity or species richness. However, the results from this long-term experiment do not agree with these conclusions. Addition of the growth limiting nutrients did not lead to an increase in biodiversity (richness) but to a decrease when high rates of slurry were applied in this experiment. Therefore N:P ratio in grass cannot fully explain totally the observations made in this experiment.

Agrostis tenuis and *Holcus lanatus* in UFCTRL had higher concentrations of total N% and P% than the sown species *Lolium perenne.* This indicates that the invading species could grow better in soil with N-limitation and P-limitation (UFCTRL: without fertilizer application for 32 years).

In addition, there was a positive linear relationship between N% and P% in the dominant species (Figure 4). Although the application of high rates of pig slurry increased the concentrations of N and P at the same time, the increase in P% in the herbage was higher than that of N%. Therefore, N:P ratios of three main species in Pig200 were the lowest compared to the other treatments, although Figure 4 shows that the concentrations of N and P in the three main species were not highest in Pig200. The data also show that *Agrostis stolonifera* had a greater ability to take up N and P from the soil than *Lolium perenne* or *Poa* spp. which have similar plant concentrations of N and P. It could also be interpreted that for a given N level, *Agrostis stolonifera* takes up less P than do the other two species.

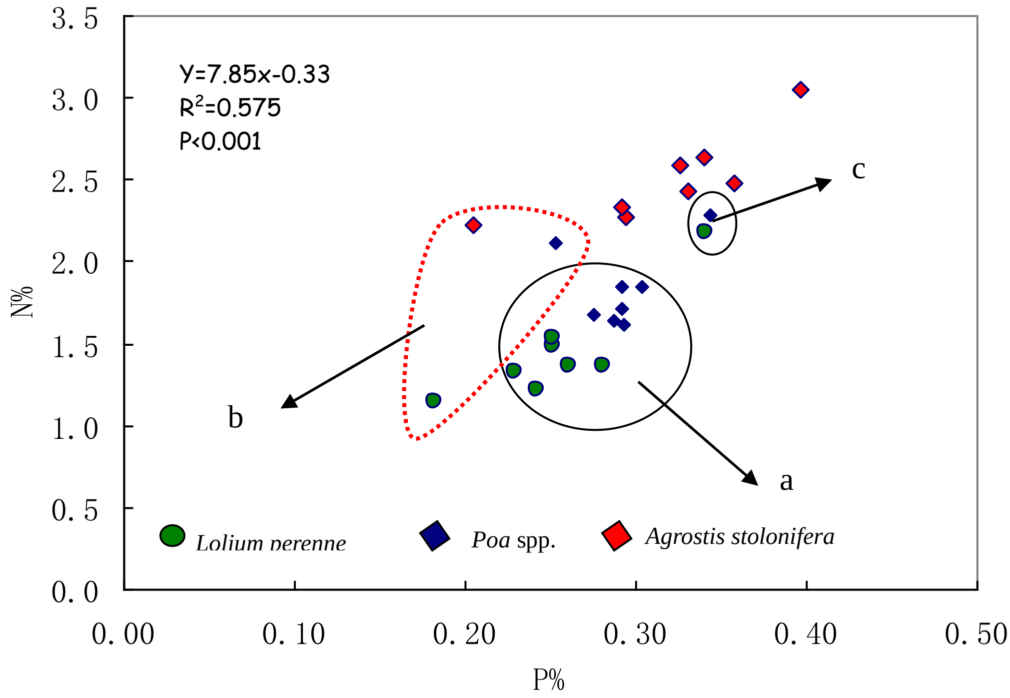

Figure 4 The relationship between N% and P% in the three main species and the patterns of N and P distribution in different treatments. a: N% and P% in *Lolium perenne and Poa* spp. in different treatments excluding UFCTRL and Cow200; b: N% and P% in three main species in UFCTRL; c: N% and P% in *Lolium perenne* and *Poa* spp. in Cow200

Other elements were also measured in the herbage including Mn, Cu, Zn, Fe, K, Na, S, P, and C (data not shown). The contents of sodium (Na) and potassium (K) in the herbage showed similar trends to the results published in 1987 by Peter Christie. There was a significant negative correlation between Na% and K% (r = 0.801, P<0.001). The content of sodium in *Lolium perenne* was higher than that in *Agrostis stolonifera*, *Poa* spp. and the remaining mixture of other species in all treatments. The concentration of potassium in *Lolium perenne* showed the opposite trend to sodium, perhaps due to antagonism in uptake between potassium and sodium (Figures not shown).

Copper (Cu) and zinc (Zn) are usually added to animal feeds in order to increase feed efficiency and to control dysentery. These two elements in the diet are excreted to pig and cow slurry. The concentrations of Zn and Cu in the main species were determined to understand whether high amounts of Cu and Zn accumulate in grass after cow and pig slurry application for 35 years. The results show that *Agrostis stolonifera* contained higher contents of Cu and Zn than did *Lolium perenne*. The contents of Cu and Zn increased with increasing application rate of slurry, and reached the highest values in Cow200 for copper (11.8 mg kg^{-1}) and in Pig200 for zinc (41.1 mg kg^{-1}) in *Agrostis stolonifera* (data not shown). Copper may be toxic to certain breeds of sheep at concentrations higher than 10 mg kg^{-1}. This indicates that long-term application of high rates of slurry to grassland could pose a potential risk of Cu toxicity to animal health.

Interestingly, the highest herbage contents of Mn occurred in *Agrostis tenuis* and *Holcus lanatus* in UFCTRL (196 mg kg^{-1} and 220 mg kg^{-1} respectively). The concentrations of Mn in *Agrostis stolonifera* were much higher than in the other main species across all treatments except for plots without fertilizer and decreased with increasing application rates of cow slurry. In the case of the sown species *Lolium perenne*, the maximum concentration of Mn appeared in the treatment without fertilizer (145 mg kg^{-1}) and there were minimum concentrations of Mn at high application rates of cow and pig slurries (45 mg kg^{-1} and 34 mg kg^{-1} respectively). We do not have enough data on manganese in slurries and soil to explain fully this trend of Mn in the three main herbage species but Mn availability may be related to the effects of the treatments on soil pH.

Other experience in the United Kingdom

- *Travelling in Northern Ireland*

When I stayed in the UK, I was also involved in some field experiments on white clover with Dr Scott Laidlaw's research group. This gave me more opportunity not only to learn some useful sampling methods in grassland, but also to visit numerous farms around Northern Ireland. Those farms are located in different parts of Norhtern Ireland, for example farms around the areas of Coleraine, Londonderry, Omagh and Downpatrick as shown on the map to the right. I really enjoyed doing this kind of field work to know the grassland landscape in Northern Ireland and to experience the nature. I believe that I would remember forever what I felt when the grassland view in countryside and Atlantic Ocean near the north coast of Northern Ireland appeared in my eyesight for the first time.

- *Trip to Dublin - the capital city of the Republic of Ireland*

I had a one-day trip to Dublin during my visit to Belfast. The National Gallery, Trinity College and the Royal Irish Academy are the main places I visited. However, I was really interested in another small organization, Information on the Environment (ENFO). People can get some information free of charge about the changes in global climate, the greenhouse effect, air-water-soil pollution etc. There are some pictures and cartoons to show children what has happened to the environment. It is very good to keep people informed about our environment and protection of the environment, especially children.

- *Three days' holiday at Oxford University*

The travelling Fellowship of the Stapledon Trust originally included some travel expenses in the UK, but the funds were spent on my UK work permit. When a friend of mine who works in Oxford University as a postdoctoral research worker invited me to his university, I decided to make a visit at my own expense. I and my supervisor (Peter Christie) recognized that the trip to Oxford University might be the only opportunity in my life. I looked around many colleges in Oxford University. I have to admit that Oxford University is one of the most famous universities in the world. I love the traditional architecture in the campus of this oldest university. This trip was very significant for me during my visit to the UK.

Plans for follow-up from the Fellowship

- Stoichiometry research in grasslands in China
- To investigate the mechanisms of the intercropping of white clover and ryegrass in grassland using plant physiology and molecular biotechnology techniques.
- A good relationship was built up further between my research centre and the Agri-Food and Biosciences Institute in Belfast. I would like to do some research in the field of plant nutrition in collaboration with Dr Peter Christie and Dr Scott Laidlaw in the future.

We anticipate publishing several journal papers based on my project when all of the data have been collected.

Acknowledgements

I really appreciate the excellent opportunity that the Stapledon Memorial Trust Travelling Fellowship provided me to do some research work in the UK. It is more important for me to have this experience working and living in Northern Ireland and to make friends with local people. I am very grateful to Dr Peter Christie and Queen's University Belfast and DARD for fully supporting me with working facilities and library facilities, and to Dr Scott Laidlaw for providing the expertise to train me in the identification and separation of the dominant plant species and laboratory facilities. Thanks are also due to Elizabeth Anne Wasson, Aaron Carrick, Margaret Wallace and Dr Ben Simon for their kind help during my visit to the UK.

References

Christie P 1987. J. Agric. Sci. Camb. 108: 529-541
Elser JJ et al. 2000. Ecology Letters 3: 540-550.
Guswell S et al. 2005. Oikos 109: 71-80
Krebs C J 1999. Ecological methodology. Benjamin/Cummings.
Naeem S et al. 1994. Nature 368: 734-737.
Ricklefs RE & Schluter D (Eds) 1993. Species diversity in ecological communities. University of Chicago Press.
Tilman D 1996. Ecology 77: 350-363.