

# **Soil properties and their influence on grassland production under low input and organic farming conditions**

## **END OF PROJECT REPORT**

**RMIS 4478**

### **Authors**

---

C. Leonard<sup>1</sup>, G. J. Mullen<sup>2</sup>, N. Culleton<sup>3</sup> and J. Breen<sup>2</sup>

<sup>1</sup> Walsh Fellow, Department of Life Sciences, University of Limerick.

<sup>2</sup> Department of Life Sciences, University of Limerick.

<sup>3</sup> Teagasc, Johnstown Castle, Wexford.

### **Johnstown Castle Research Centre Wexford**

Part of this work was conducted under the auspices of the Teagasc Walsh Fellowship Programme.

**January 2006**



**AGRICULTURE AND FOOD DEVELOPMENT AUTHORITY**

---

Teagasc Headquarters, Oak Park, Carlow

## TABLE OF CONTENTS

<b>SUMMARY .....</b>	<b>1</b>
<b>INTRODUCTION .....</b>	<b>2</b>
<b>MATERIALS AND METHODS.....</b>	<b>3</b>
<b>Soil and site sampling and analyses .....</b>	<b>4</b>
<b>Rowell, (1994); Amato and Ladd, (1988) .....</b>	<b>5</b>
<b>Botanical composition analysis .....</b>	<b>5</b>
<b>Grassland management and production analyses.....</b>	<b>5</b>
<b>Data analysis for statistical and ordination purposes.....</b>	<b>6</b>
<b>RESULTS AND DISCUSSION .....</b>	<b>7</b>
<b>Overview of the sixteen study farms .....</b>	<b>7</b>
<b>1. Site and soil physical and chemical properties .....</b>	<b>7</b>
<b>2. Soil biological properties.....</b>	<b>7</b>
<b>3. Grassland botanical composition.....</b>	<b>8</b>
<b>4. Grassland production .....</b>	<b>8</b>
<b>5. Nutrient management .....</b>	<b>8</b>
<b>6. Soil disruption .....</b>	<b>9</b>
<b>7. Grazing practices .....</b>	<b>9</b>
<b>RELATIONSHIPS AND PATTERNS INFLUENCING GRASSLAND PRODUCTION.....</b>	<b>9</b>
<b>1. Many significant relationships were as expected .....</b>	<b>9</b>
<b>2. Soil pH and other influences work together.....</b>	<b>10</b>
<b>3. Soil biological activity may indicate production.....</b>	<b>10</b>
<b>4. The role of soil organic matter .....</b>	<b>13</b>
<b>CONCLUSIONS AND RECOMMENDATIONS.....</b>	<b>15</b>
<b>ACKNOWLEDGEMENTS .....</b>	<b>16</b>
<b>REFERENCES .....</b>	<b>17</b>

## SUMMARY

This project set out to identify soil properties that most influence grassland production under low mineral nitrogen input conditions. Sixteen farms were selected in Counties Limerick and Clare and the soil sampled. Soil physical and chemical characteristics and soil biological aspects involved in the carbon and nitrogen cycles were studied in the laboratory. Nutrient additions to farms as well as the nature of grazing by livestock (numbers, types of grazing animals, grazing practices), grassland management, and production from the farms were recorded.

Farms varied from intensively conventionally managed, through varying degrees of organic management, to very low nutrient input management. Highest mineral N applications ( $174 \text{ kg ha}^{-1}$ ) were applied on a conventional farm, and highest organic N ( $56 \text{ kg ha}^{-1}$ ) applications on an organic farm. Annual energy production ranged from 12 to  $66 \text{ GJ ha}^{-1}$ . Soil physical and chemical properties were within the expected range, and all farms exhibited soil biological activity, each having the measured C and N cycling capabilities to some degree.

Statistical and ordination analyses using the SPSS and MVSP computer packages identified significant relationships and helped patterns emerge. Significant relationships were found, many as expected. Production was positively correlated with soil pH, recent soil disturbance (such as ploughing and reseeded), and plant-available soil magnesium content, as well as with P and K inputs, soil depth and plant-available soil calcium. In contrast, production was negatively correlated with root mass and pasture ageing.

Farms in which either mineral or organic nitrogen fertilizer had been applied were more productive. Surprisingly, production was lower in farms exhibiting rapid nitrogen mineralization as indicated in soils amended with the amino acid arginine in the laboratory. This was unexpected because it has often been assumed that organic farming would require more, not less, 'soil biological activity'. These laboratory results mean that if organic nitrogenous compounds were either added or available, N would be released more rapidly by less productive soils. However, less productive soils are not necessarily more biologically active under normal field conditions where substrates may be limited.

Although some measured variables were more important for production than others, all of the important factors were significantly related to each other, and worked together rather than in isolation. For example, while a strong relationship between production and soil pH was observed, high and low production levels were achieved at the same pH values and so additional influences were implicated. Three groups of farms (high, average and low production) became obvious by putting those factors that significantly influenced *in vitro* nitrogen mineralization together in one principle component ordination diagram. The diagram confirmed that the grassland production environment is created by a number of contributory factors acting together, amongst which N-mineralization is key.

When organic matter was added as arginine (an amino acid) to soil samples in the laboratory, the resultant *in vitro* N-mineralization was significantly more rapid in soil taken from unfertilized and undisturbed pastures. In the absence of either fertilizers or disturbances, soil organic matter may have senesced forcing bacteria to use the more readily available arginine, which was added in the laboratory, for energy instead. Fundamental research literature supports this hypothesis. Results here also imply that unfertilized and undisturbed soils lack the substrates that would be required for either N-mineralization or N-immobilization *in situ*, which could explain their poor production levels. However, the organic carbon pool measured in this study encompasses large complex organic molecules as well as small molecules that could be taken up by microorganisms for energy and growth. Likely effects of available C on soil N mineralization and N immobilization should be thoroughly investigated.

Total soil organic carbon (TOC) content ranged from 3 to 9%. These levels are high in comparison with levels reported in comparable studies. No significant relationships were found here between TOC content and either the microbial biomass or its biological activity, in contrast to comparable studies. The TOC content may be below a limiting threshold elsewhere but not in this research. If limiting TOC content thresholds exist, they should be identified and the knowledge applied so that adequate organic matter is managed and maintained, and so that soil nutrient-cycling activities are not constricted.

Whilst grassland management practices are likely to influence soil fertility through combined C and N effects, there was a strong indication in this research that one of the soil parameters recorded, microbial activity as arginine ammonification, could predict grassland production potential reliably. If this finding can be validated by further study, microbial activity as arginine ammonification may form the basis for a rapid soil test to enable farmers adjust their grassland management practices so that both nitrogen mineralization and nitrogen fertilizer applications can be optimized.

## INTRODUCTION

Conventionally, grassland production potential is determined by the soil and sward, supported by fertiliser inputs. Well-considered nitrogen (N) application rates are recommended for silage and grazing grounds, respectively (Coulter, 2001), and soil tests are available to assess lime and phosphorus (P) and potassium (K) fertiliser requirements, such that optimal production can be assured.

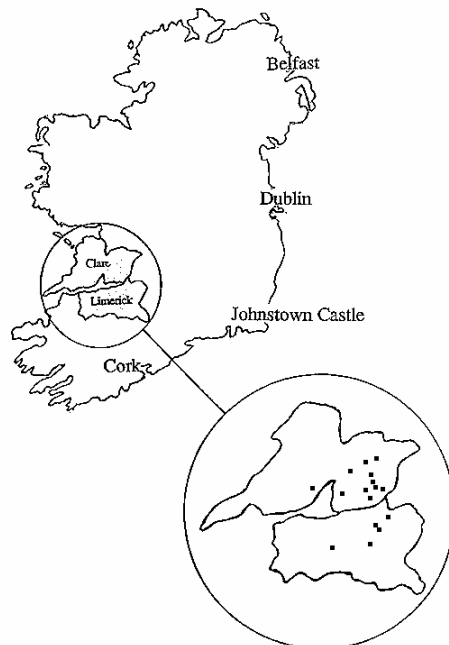
In organic grassland however, or in any situation where mineral N is not applied, production largely depends on the biological cycling of N from legumes and from organic matter either present in soil or inputted from

excreta, slurries or manures. While the role of atmospheric N fixation within legume root nodules is acknowledged, it is still not fully understood how legume N becomes available to grasses in the sward, and, in practice, N mineralization is not specifically ‘managed’ (Newton, 1993). Biological analysis should be done for organic farming purposes (Lampkin, 1990), but researchers have encountered methodological difficulties, and, for example, soil N mineralization potential has not yet been satisfactorily predicted (Burket and Dick, 1998).

The objective of this study was to identify those soil properties that most influence grassland production under organic farming conditions, amongst which the soil biology was expected to play an important role. Because many soil properties, vegetation characteristics and management practices are likely to influence soil fertility, and probably affect each other, they are considered together in this study.

## MATERIALS AND METHODS

Based on local advice (Kelly, 1994) a number of grassland farmers interested in organic farming were approached in Counties Limerick and Clare. Twenty farms were selected for soil and management analyses. Subsequently, four farms for which management data could not be obtained were dropped from the study. The remaining sixteen farms were encoded alphabetically for confidentiality purposes and their approximate location is shown in Figure 1.



**Figure 1. Location of study farms in Counties Limerick and Clare.**

## Soil and site sampling and analyses

During summer 1995, soil was sampled at random avoiding ditches gates and walkways and taken to the laboratory from a field which the farmer and the authors felt represented farm production in each case. At each of ten randomly selected points, cylindrical cores, 12.2 cm long and 1.52 cm in diameter, were removed using a Teagasc-type sampler, for root mass analysis. At five of those points, two clods were removed undisturbed and carefully bagged. Of these ten clods: three were used for determining soil bulk density using a modification of the clod method of Blake (1965a); two were disrupted and sieved for the determination of physical and chemical parameters; and five were frozen at  $-18^{\circ}\text{C}$ . These five were later defrosted and soil from the 3 to 5cm depth taken, bulked and shaken vigorously in a plastic bag for five minutes prior to sub-sampling for immediate biological analyses. All clod samples extended from the soil surface to 10cm depth.

In brief, site attributes were either read from soil maps or measured *in situ* and soil physical and chemical properties were tested as described in the literature. Microbial biomass was estimated from both the rate of respiration after glucose addition and the amount of nitrogenous material that reacted with ninhydrin. Total viable bacterial populations were measured on nutrient agar. Bacteria that could break down carbohydrates (cellulose and starch) or nitrogenous compounds (protein) and an amino acid (arginine) were counted by inoculating soil dilutions onto media containing those substrates. Biological activity arising from intact microbial cells was measured as the rate at which ammonia was produced when arginine was added to soil samples incubated in the laboratory (arginine ammonification). Urease enzyme is synthesised by microbial cells but is released into the soil environment where it is stabilised and cell-free. Urease enzyme activity was measured as the rate ammonia was produced in urea-amended soil incubated in the laboratory. Analytical methods and references used are summarised in Table 1.

**Table 1: Details of soil and site analysis**

<b>Parameters</b>	<b>Basis for methods used</b>
<b>Site related</b>	
Altitude, slope, aspect	<i>In situ</i> measurement
Soil series, depth, percent clay, silt to clay ratio	Finch & Ryan (1966); Finch (1971)
<b>Physical and chemical</b>	
Root mass, bulk density, total porosity, aggregate ratio, aggregate stability; Soil pH; percent organic carbon (OC), soil nitrogen; plant-available contents of soil Mg, K, Ca; Cation exchange capacity	Brink et al., (1960); Blake (1965 a & b); Kemper (1965); Byrne, (1979); Rowell, (1994) Killion, (1996)
<b>Biological</b>	
Biomass as substrate induced respiration and ninhydrin reactive nitrogen	Rowell, (1994); Amato and Ladd, (1988)
Bacterial populations	Oxoid ® and other media
Arginine ammonification Urease enzyme activity	Alef, (1995); Klein and Koths, (1980).

### **Botanical composition analysis**

Grassland botanical composition was measured in February 1998. A quadrat of vegetation was cut close to soil level, bagged and brought to the laboratory where the species were identified and separated into bundles for drying overnight at 100°C. Dry weights of each species and total vegetation were obtained, and the botanical diversity of the grassland was recorded as the number of species found.

### **Grassland management and production analyses**

In 1997, each farmer was interviewed to obtain information in regard to management of the farm for the previous five years. Whether the farm had been organically managed, and for how long, was determined. Lime or fertiliser additions to the entire farm and the sample fields were recorded. Amounts and types of livestock on the farms, livestock bought and sold, fodder produced on the farm and fodder bought and sold, and milk produced on the farm, were quantified. How the sampled fields had been grazed (i.e. the numbers and types of animals and the rotation used) was investigated. Whether the farm or the sampled fields had been reseeded or their soil disturbed in any way, was also ascertained, so that pasture age could be estimated.

The numbers, types and livestock unit equivalents of grazing animals, likely excreta deposited, and the periods of times grazed and rested were used in different ways to describe grazing intensity, treading, and excretal N, P and K deposited on the sampled field in the year prior to soil sampling, so as to take the effects of grazing on soil characteristics into account.

Grassland production was assessed in terms of the amount of energy needed to produce each item during 1996-1997. For this, the metabolisable energy equivalents (MEE) of each unit of livestock, milk and fodder that had been produced on the farm in that year were sought from ADAS (1984), Evans et al. (1990), Frame (1992) or Cooper (1995), as appropriate. The quantity of a unit produced multiplied by the energy required for its production (MEE) was entered into the formula, adapted from Frame (1992), below. Thus the grassland production required to produce livestock, offspring and dairy products is given by:

$$P_g = (L+X - I - N) / A$$

where

$P_g$  = Grassland production (GJ ha<sup>-1</sup>)

L = MEE required to produce livestock, offspring & dairy products (GJ);

X = MEE exported from the farm in sold grassland fodder crops (GJ);

I = MEE imported as purchased ruminant feedstuffs (GJ);

N = MEE used from farm but non-grassland fodder crops (GJ)

A = Area of grassland (ha)

.

### **Data analysis for statistical and ordination purposes**

An Excel database was created as a basis for study. Frequency histograms were set up using the Statistical Package for the Social Sciences, SPSS (Norusis, 1992; SPSS, 1999) to check the normality of the data. As the data were not normally distributed, the nonparametric Spearman's rank correlation analyses were carried out using SPSS to establish any statistically significant relationships within variable pairs. Patterns were sought using Principle Component Analyses to create ordination diagrams of the dataset and subsets thereof through MVSP, the MultiVariate Statistical Package (Kovach, 1998).

The materials and methods used in this research are given in more detail elsewhere (Leonard, 2001).



## RESULTS AND DISCUSSION

### Overview of the sixteen study farms

#### 1. Site and soil physical and chemical properties

Six of the study farms were on Elton, four on Patrickswell, two on Gortaclareen, and one each on Ballylanders, Baggotstown, Cooga, and Kilfergus, soil series. All were lowland farms, and except for one reportedly wet farm could be described as well drained. Soil physical and chemical properties are summarised in Table 2.

<b>Table 2: Soil physical and chemical properties of the study farms, summarised</b>				
<b>Soil physical properties</b>	<b>Min.</b>	<b>Max.</b>	<b>Mean</b>	<b>SD</b>
Root mass, (estimate in kg ha <sup>-1</sup> )	36941	122404	78294	28119
Bulk density (g cm <sup>-3</sup> )	0.98	1.27	1.10	0.08
Particle density (g cm <sup>-3</sup> )	2.13	2.46	2.35	0.08
Pore space (%)	47.00	60.00	53.25	3.69
Ratio of small to large aggregates (ratio of <1 mm to >1 mm)	1.29	10.95	3.97	2.41
Water stable aggregates (%)	63.17	88.94	81.41	6.47
<b>Soil chemical properties</b>				
Soil pH	4.91	7.15	5.90	0.66
Organic carbon (%)	3.19	9.61	6.00	1.58
Soil nitrogen (%)	0.31	0.72	0.51	0.11
C:N ratio	9.38	17.21	12.09	1.84
Plant-available soil P (mg l <sup>-1</sup> )	1.4	12.90	5.36	3.40
Plant-available soil K (mg l <sup>-1</sup> )	10.00	250.00	93.59	71.77
Plant-available soil Ca (mg l <sup>-1</sup> )	310.00	13500.00	3989.06	3733.37
Plant-available soil Mg (mg l <sup>-1</sup> )	60.00	350.00	214.38	80.74
CEC (cmol (+) kg <sup>-1</sup> )	5.92	18.72	12.07	3.69
Polysaccharide content (mg ml <sup>-1</sup> )	8.05	55.97	31.17	14.27

Generally, soil conditions were as would be expected for Irish grassland. However, some farms at relatively high or low soil pH values may be disadvantaged, and plant available soil potassium content is relatively low overall. Root mass values per hectare had been extrapolated from the small cores taken and were far higher than in comparable studies. Nevertheless, root mass may have accumulated in the older pastures studied.

#### 2. Soil biological properties

Each sampled field had measurable amounts of: microbial respiration in response to added glucose; nitrogenous biomass; bacterial populations; bacterial heterotrophs capable of breaking down the carbonaceous (cellulose, starch) and nitrogenous (protein, arginine) substrates that may be present in soil (Richards, 1987); and soil urease enzyme activity, indicating that each of the farms in the study had the biological capabilities needed for

N and C turnover. However, biological parameter values varied widely. For example, ammonia was produced in ten of the sixteen soils amended with arginine in the laboratory, which meant that nitrogen mineralization was taking place in those samples. Negative values for ammonia-N, representing nitrogen immobilisation, were recorded *in vitro* in soil from five farms.

Norms for soil biological attributes are not yet established (Sparling, 1997). However, apart from negative arginine ammonification values that are rarely reported, the soil biology values obtained here were similar to those in the literature.

### 3. Grassland botanical composition

Swards were grassy for the most part, having between 30 and 90% grass content, dominated by *Agrostis* and *Holcus* species followed by *Lolium* which was the most constant of species found, being recorded on all but one farm. The mean clover content was 8%. Botanical diversity ranged from 4 to 11 species, and was much lower than the minimum of 25 species found in lowland grasslands in Co. Limerick thirty years before by O'Sullivan (1968). It is likely that the more intensive management practiced nowadays has influenced grassland botanical diversity.

### 4. Grassland production

Grassland was the main farm enterprise with farms variously grazing goats, sheep, cattle and dairy goats and cows, some with mixed grazing. The year's production ranged from 12 to 66 GJ ha<sup>-1</sup>, being highest in three 'most organic' and the conventional farm, and lowest in a farm practicing summer grazing only.

### 5. Nutrient management

In 1997, four farms had been without fertilisers for many years, four were in conversion to organic farming, seven had been managed organically for at least two years, one farm was managed conventionally, and a wide range of nutrient inputs had been made overall (Table 3).

Table 3 : Fertiliser N, P and K (kg ha <sup>-1</sup> yr <sup>-1</sup> ) applications during the period 1992-1997, summarised					
Nutrient source	Element	Min.	Max.	Mean	SD
Mineral fertilisers (kg ha <sup>-1</sup> y <sup>-1</sup> )	N	0	174.1	11.8	43.4
	P	0	44.2	3.5	11.0
	K	0	94.6	7.3	23.5
Organic fertilisers (kg ha <sup>-1</sup> y <sup>-1</sup> )	N	0	56.3	9.4	16.1
	P	0	43.9	11.8	12.4
	K	0	131.4	20.2	35.7

Fertiliser applications were considered over the six-year period from 1992 to 1997 to span nutrient management transitions that farms might have undergone. Although mean values suggest that low levels of fertilisers had

been applied to many farms, in reality, mineral nitrogen was applied annually ( $174 \text{ kg ha}^{-1}$ ) on only the conventional farm, and was applied to only two others, at 29 and  $56 \text{ kg ha}^{-1}$  prior to their conversion to organic management. The highest organic N ( $56 \text{ kg ha}^{-1}$ ) applications were made on an organic farm. Eleven farms had applied lime.

## **6. Soil disruption**

As mechanical disturbances affect soil organic matter content, introduces air and increase biological activities such as N mineralization (Whitehead, 1995), whether soils had been disrupted in any way was ascertained. Five fields had previously been used for arable crops, one had been ploughed to remove ferns, and another had undergone regular chain harrow use to remove dead vegetation. All five fields had been reseeded. A sixth field was reseeded without mechanical disruption of the soil by scattering seed from the back of a tractor. All six had applied a traditional *Lolium* / *Trifolium* seed mixture.

As expected, the field that had been ploughed most recently, three years before sampling, had the lowest root mass. Overall, root mass correlated positively ( $P < 0.05$ ) with the length of time since soil disruption, probably accumulating in undisturbed soils over time.

## **7. Grazing practices**

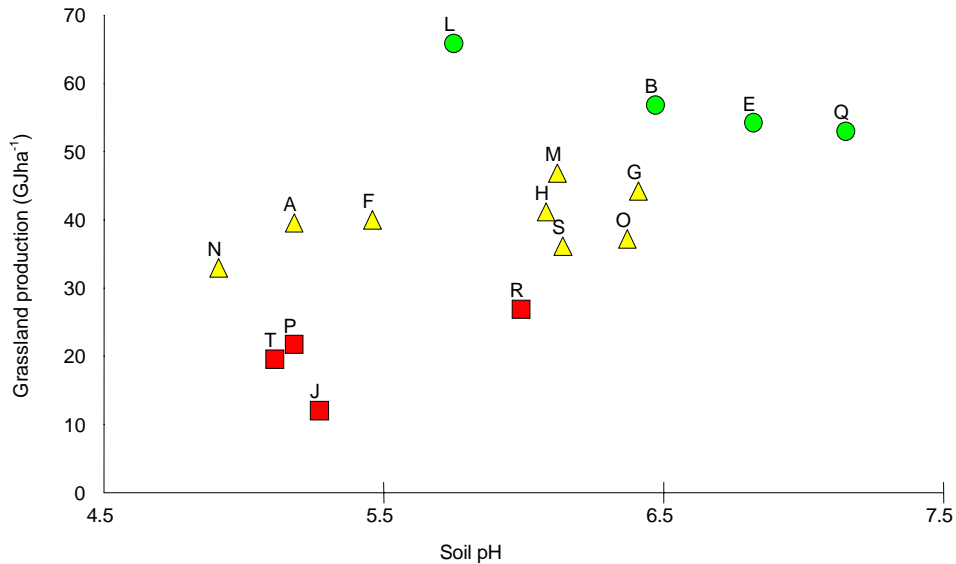
Most of the study farms were block grazed, field by field. However, strip grazing occurred on two intensive dairy farms, and two farms were set stocked. The sampled fields were exposed to different estimated intensities of grazing, treading and excreta deposition throughout the year before soil sampling occurred.

# **RELATIONSHIPS AND PATTERNS INFLUENCING GRASSLAND PRODUCTION**

## **1. Many significant relationships were as expected**

Many of the significant relationships found between grassland production and variables describing the grassland management and soil properties were as expected; all are described here. Production was positively ( $P < 0.01$ ) correlated with soil pH (Fig.2), recent soil disturbance, and plant-available soil magnesium content, as well as with ( $P < 0.05$ ) N, P and K inputs, soil depth and plant-available soil calcium. This shows that fertiliser inputs, however slight, and whether mineral or organic, led to increased grassland production. In contrast, the effects of larger quantities of vegetation dry weight and root mass, and of pasture ageing, were negative ( $P < 0.01$ ). It can be presumed that fresher pastures were best because root mass content was a negative influence that increased with pasture age ( $P < 0.05$ ).

Some important relationships and patterns that emerged are shown in Figures 2, 3 and 4. Farms are coded alphabetically, and high, medium or low production farms are represented by green circles, yellow triangles, and red squares, respectively, here.



**Figure 2. Grassland production (GJ ha<sup>-1</sup>) in relation to soil pH**

## 2. Soil pH and other influences work together

Although grassland production and soil pH are strongly correlated, the graph of soil pH in relation to grassland production is not linear, and farms are scattered such that the highly productive farm L and low production level farm R have very similar soil pH values. While there is no doubt that soil pH is influential, it can be inferred that it is not the sole determinant of grassland production, and other influences are likely.

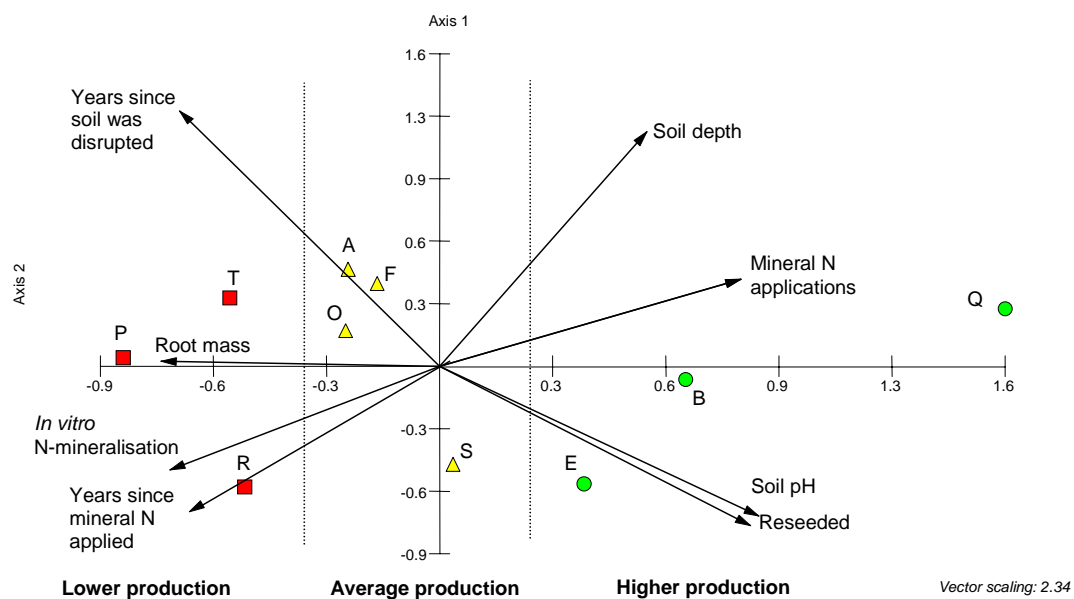
## 3. Soil biological activity may indicate production

Surprisingly, production was lower ( $P < 0.01$ ) with more rapid ammonia production from arginine, or *in vitro* nitrogen mineralization, a biological activity. This contradicts earlier suggestions (Bolton et al., 1985) that more biological activity should accompany organic farming. Instead, soils that either mineralised or immobilised nitrogen at higher rates were from less productive farms, and soils from productive farms were not extreme in either N-turnover step. While it is not known whether the ammonia nitrogen that might be released *in situ* from more rapid mineralization activity is used by non-productive species or lost through volatilisation, it can be concluded that optimal organic matter nitrogen turnover rates should neither restrict nor accelerate mineral N-provision, but should 'tick over', probably at rates commensurate with grass growth.

While soil from productive farms had low levels of N-turnover activities, values varied considerably across all farms and it became clear after careful

study that N- turnover processes were influenced by other variables. For example, *in vitro* N-mineralization correlated significantly ( $P < 0.05$ ) with root mass and with the interval since mineral nitrogen applications, being more rapid in permanent and unfertilised pastures.

To visualise how variables interact, all of the factors that correlated significantly with *in vitro* N-mineralization were entered with it in a principal component ordination diagram (Figure 3). Principal component analysis (PCA) is used mainly in ecology and is a way to identify the most important contributory factors from amongst a number of variables that describe sites, examined together. The researcher chooses which sites and variables to enter and, if chosen well, the patterns that emerge will help to explain how variable values relate to each other, and to their environment. Essentially, PCA takes a cloud of data points and rotates it through a series of axes two by two to explain the data entered, at each consecutive stage showing which factors are influential, and to what extent. The percentage difference between sites (or farms) explained by the data is given as part of the PCA output (Kovach, 1998; Legendre and Legendre, 1998; Palmer, 2001).



**Figure 3. Pca ordination diagram implemented with those soil and management variables that correlated significantly with *in vitro* n-mineralization, for the ten farms in which n-mineralization was recorded in arginine-amended soil in the laboratory.**

Three axes have been extracted by this PCA ordination. Axes one and two, shown, account for 70%, all three account for 85% of between-farm variation. The ten farms in which *in vitro* N-mineralization was recorded are scattered across Fig. 3 according to the soil and management variables used to depict the overall farm environment. From left to right, farms P, R and T have lower levels of production, older pastures, higher levels of root mass, and higher rates of *in vitro* N-mineralization than other farms. Mineral nitrogen applications have not been made to these farms for some time. In the mid section, farms A, F, O and S have average production levels. To the right, farms E, B and Q have deeper soils, higher soil pH values and

*their grassland was reseeded. Mineral nitrogen has been applied on these farms, in particular to Q, the conventional farm. Farms in this section are highly productive.*

Because Figure 3 is based on the *in vitro* N mineralization rate and on variables related to it, it includes only those ten farms in which *in vitro* N-mineralization was recorded.

The extraction of only three axes, that together account for 85% of farm variation means that *in vitro* N-mineralization and related variables are important variables, and all of the variables entered in the diagram help to describe and differentiate each farm environment.

The labels used to describe each variable in Figures 3 and 4 are self-explanatory. Variables extend towards farms in which their values are highest, and relative positions of farms and vectors are as expected from statistical analysis, and can be readily explained here too. For example farm Q, the most conventionally farmed, is located beyond the vector for mineral N applications, whereas farm R in which mineral nitrogen had not been applied for a number of years is in the opposite sector. Absence of either soil disturbances or mineral nitrogen additions, and more root mass typify less productive farms to the left of Figure 3. Opposite environments have higher soil pH, greater soil depth, and tend to have been disrupted and reseeded, to have mineral N added, and to be more productive.

A clear pattern emerged in that productive, average and poorly productive farms occurred in separate sectors, highlighted here by dotted lines overlaid on the original PCA diagrams. The interdependence of all contributory factors is obvious, and it is clear that each factor acts in concert with others, together contributing to the overall grassland production environment.

As N-immobilisation occurred in five farms, and as insufficient soil was available to obtain an arginine ammonification value for farm G, two further PCA ordinations were implemented. The first was designed to see if the pattern emerged from farms in which nitrogen was immobilised, the second to test whether, without using arginine ammonification values, the factors related to it could predict production reliably.

In both instances similar patterns were observed such that productive, average and less productive sectors could be discerned. The second example (Fig.4) is shown here.



likely that grassland management contributed to the relatively high TOC content (3-9%) found. For example, higher TOC content levels corresponded significantly ( $P < 0.05$ ) with the interval since mineral nitrogen applications, and TOC content was lowest on the conventional farm.

In contrast to three comparable studies conducted by Alef and Kleiner (1987), Lin and Brookes (1999) and Haynes and Tregurtha (1999), no significant relationships were found here between TOC content and either the microbial biomass or its biological activity. However, in the three reports mentioned, relationships between TOC content and microbial activity were linear in soils containing less than 5% TOC, but were not established as linear in those studies when soils contained 6% TOC content (the mean TOC content found in the present study) or above. At relatively low TOC content levels, the soil biology which is contained within soil organic matter may have been squeezed proportionately, giving rise to significant relationships, and TOC content may be below limiting thresholds elsewhere. Loveland and Webb, (2003) reviewed numerous studies indicating that a critical organic matter threshold exists below which soil quality, soil physical properties and crop nutrition deteriorate, but concluded that quantitative evidence was lacking. Similarly, results and comparisons made here suggest that organic carbon thresholds may limit soil biological functions. As recommended by Loveland and Webb (2003), potentially critical threshold TOC levels should be ascertained. Such awareness would inform grassland management practice in order to maintain soil organic matter levels and not jeopardise future soil quality, physical properties, nutrient cycling activities, and crop nutrition.

In contrast to TOC content, known bacterial behaviour and observations in this study suggest that available carbon (AC) plays an important role. Fundamentally, many soil bacteria can use carbohydrate, fat or protein as substrates (Richards, 1987). However, most bacteria use glucose first before sacrificing other molecules such as amino acids for energy (Cunin et al., 1986). Observations of the farms in this study indicate that the soil biology behaved according to those principles, as follows: more heavily grazed soil had significantly higher populations of bacteria capable of digesting cellulose; cellulose has probably been macerated into soil by treading. Fields that were “more rested” had more bacteria likely to use arginine, an amino acid: it’s presumed that cellulose and its breakdown product glucose are not so readily available under rested conditions. Finally, higher root mass levels, where carbon may be locked up rather than readily available, were associated with more arginine ammonification and so had higher N-mineralization rates. It is likely that grazing intensity and root mass impacted on bacterial populations and activity in the study farms through a common mechanism, i.e. microbial substrate availability. Just as AC use for energy might reduce N mineralization activity, AC is also required by microorganisms in order to incorporate N in molecules during N immobilisation. Nitrogen ammonification and immobilisation form a continuum, that may be driven towards N mineralization when AC is in short supply, or towards N immobilisation when AC is plentiful. Nitrogen is



released from microbial biomass breakdown though, and so biomass N acts as a resource. Based on these observations it is proposed that AC content, rather than TOC content, regulates nitrogen mineralization in Irish grassland, and the senior author is currently testing this hypothesis.

Observations made in this study suggest that the pool of microbe-available substrates not only regulates the rate, but also importantly determines the extent, of N-mineralization in the field. Patterns in PCA ordinations and in significant correlations showed that farms that were less productive had undisturbed pastures, had been without mineral nitrogen applications for some time and had larger root mass levels. Under these conditions, organic carbon is likely to have senesced, and so would be less readily available to microorganisms for energy and growth. Nitrogenous organic compounds could become equally recalcitrant. Under older unfertilised pasture conditions, resources for N-mineralization *in situ* are likely to be limited, leading to the poorer production observed there.

## CONCLUSIONS AND RECOMMENDATIONS

As expected, N-fertilised farms were more productive. Also as expected, grassland production in the study was strongly positively correlated with soil factors such as pH, depth, and plant-available soil magnesium. Rapid soil nitrogen mineralization *in vitro* was a significant negative factor, and contrary to the expected requirement for more soil 'biological activity' under the low mineral N input conditions generally found in the study farms. However, a biological response to substrate added in the laboratory does not mean that soil is biologically active under normal field conditions. As recommended by Nannipieri et al. (1990), soil biological analyses should be interpreted precisely, according to what they mean.

Many of the influential factors measured correlated significantly with *in vitro* N-mineralization rates, and together describe the production environment such that farms are separable into high, average and low production, in a PCA ordination diagram. Because of interdependence amongst important soil factors, they should be analysed together to depict how the environment works as a whole. Ordination analyses can be applied successfully to this end. In particular, factors that interact with each other and show co-linearity can be incorporated together in PCA, unlike multiple regression analysis from which correlating factors would be excluded.

Soil organic carbon content was probably influenced by nutrient management but was unrelated either to grassland production or to the soil biology. Lack of correlation between soil TOC content and soil biology suggests that the TOC content of the study farms is above a limiting threshold. Further research might identify such limiting thresholds, how they vary with climatic conditions, and what management strategies might be employed to manage and maintain soil organic matter.

The study showed that the soil biology functions according to known principles of intracellular control, in that bacteria probably use preferred and available carbon substrates first, before breaking down nitrogenous organic matter, in other words before nitrogen mineralization. Furthermore, given available carbon as a substrate, the mineralised or mineral N present in the soil may be readily immobilised. As soil from older unfertilised pastures used arginine more rapidly, thereby demonstrating more rapid *in vitro* N-mineralization, such pastures are likely to have limited substrate resources for either N-mineralization or N immobilisation to occur, and would be less productive. Suitable assays should be developed to monitor AC concentration in soil, and the effects of AC on N-supply in soil should be investigated. Importantly, AC presence is most likely governed by whether the soil has been either externally N-fertilised or has been physically disturbed, and so grassland management itself will determine both biological outcomes below ground, and associated soil fertility levels.

It can be concluded that arginine ammonification, on which *in vitro* N-mineralization rate measurements are based, is a reliable albeit negative grassland production indicator. Its development and adaptation as a rapid soil fertility assay is warranted and is being pursued in separate research. The partnership, evident here, between N fertiliser management and soil C and N cycling activities should be further elucidated.

## **ACKNOWLEDGEMENTS**

We would like to thank the following people: O. Kehelly whose quest for an identification of soils suited to organic farming sparked off this project; P. Kelly who identified farms suitable for study in Counties Limerick and Clare; P. Kelly, M. Leonard, P. McNamara, M. O'Dwyer and H. Robson for their advice on interview content and design. We are especially grateful to the thirty farmers who allowed access to their farms for site and soil analyses, and who gave their time generously to recount farm and management practices. We would like to thank Jackie Kennedy, Joan Leahy and Maureen Davoren for technical assistance, and Fintan Barron, Annette Brennan, Noel Bridgeman, Simon Carroll, Patrick Devitt, Anita Gothard, Lorraine Herity, Brendan Hyland, Paul Lonergan, Geraldine Lyons, Lorraine McCoole, Joan Moran, Anne Mullen, Caroline Murphy, Marylyn O'Connell, Anita O'Meara, Sandra Tiernan and Mairead White, whose practical work carried out as students in the University of Limerick contributed either directly or indirectly to the work done. C. Leonard is indebted to the late L.Kieran Dunican who taught soil microbiology at UCG, and who encouraged this particular investigation at the outset.

## REFERENCES

- Abril, A., Caucas, V., Bucher, E.H.** 2001. Reliability of the in situ incubation methods used to assess nitrogen mineralisation: a microbiological perspective. *Applied Soil Ecology* 17, 125-130.
- ADAS.** 1984. Energy allowances and feeding systems for ruminants. Ref. 433, HMSO.
- Alef, K.** 1995. Nitrogen mineralisation in soils. In: Alef K., Nannipieri P. (Eds.), *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press Limited, London, pp. 234-235.
- Alef, K. Kleiner, D.** 1987. Applicability of arginine ammonification as indicator of microbial activity in different soils. *Biology and Fertility of Soils* 5, 148-151.
- Amato, M., Ladd, J.N.** 1988. Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. *Soil Biology and Biochemistry* 20, 107-114.
- Blake, G.R.** 1965a. Bulk Density. In: Black, C. A. (Ed.) *Methods of Soil Analysis, Part 1: Physical Properties*. American Society of Agronomy, Wisconsin, p. 374.
- Blake, G.R.** 1965b. Particle Density. In: Black, C. A. (Ed.) *Methods of Soil Analysis, Part 1: Physical Properties*. American Society of Agronomy, Wisconsin, p. 371.
- Bolton, H. Jr., Elliott, L.F., Papendick, R.I., Bezdicsek, D.F.** 1985. Soil microbial biomass and selected enzyme activities: effect of fertilization and cropping practices. *Soil Biology and Biochemistry* 17, 297-302.
- Brady, N.C.** 1990. *The Nature and Properties of Soils*. Macmillan Publishing Company, NY 621pp.
- Brink, R.H., Dubach, P., Lynch, D.L.** 1960. Measurement of carbohydrates in soil hydrolysates with anthrone. *Soil Science* 89, 157-166.
- Burket, J.Z., Dick, R.P.** 1998. Microbial and soil parameters in relation to N mineralisation in soils of diverse genesis under differing management systems. *Biology and Fertility of Soils*, 27, 430-438.
- Byrne, E.** 1979. *Chemical Analysis of Agricultural Materials: Methods used at Johnstown Castle Research Centre*. An Foras Taluntais, Wexford.
- Cooper, R.A.** 1995. Sheep and Goats. In: Soffe, R.J. (Ed.), *Primrose McConnell's 'The Agricultural Notebook'*, Blackwell Science, pp. 445-463.
- Coulter, B.S.** 2001. Nutrient and trace element advice for grassland and tillage crops. Teagasc IE 67 pages ISBN 1 84170 246 3 6672

- Coyne, M.S.** 1999. Soil Microbiology: An Exploratory Approach. Delmar Publishers, NY 462pp.
- Cunin, R., Glansdorff, N., Piérard, A., Stalon, V.** 1986. Biosynthesis and metabolism of arginine in bacteria. *Microbiological Reviews* 50, 314-352.
- Evans, J.W., Borton, A., Hintz, H., van Vleck, D.** 1990. *The Horse*. WH Freeman.
- Finch, T.F.** 1971. *Soils of Co. Clare*. An Foras Taluntais, Dublin. 264 pp.
- Finch, T.F., Ryan, P.** 1966. *Soils of Co. Limerick* An Foras Taluntais, Dublin 199 pp.
- Frame J.** 1992. *Improved Grassland Management*. Farming Press Books, UK, 351 pp.
- Haynes, R.J., Tregurtha, R.** 1999. Effects of increasing periods under intensive arable vegetable production on biological, chemical and physical indices of soil quality. *Biology and Fertility of Soils*, 28, 259-266
- Kemper, W.D.** 1965. Aggregate Stability In: Black, C. A. (Ed.), *Methods of Soil Analysis, Part 1: Physical Properties*. American Society of Agronomy, Wisconsin, p. 511.
- Kelly, P.** 1994. Kelly Consultants, Ennis, Co. Clare. Personal communication.
- Killion, A.** 1996. Faculty of Agriculture UCD, Personal communication.
- Klein, T.M., Koths, J.S.** 1980. Urease, protease and acid phosphatase in soil continuously cropped to corn by conventional or no-tillage methods. *Soil Biology and Biochemistry* 12, 293-294.
- Kovach, W.L.** 1998. MVSP- A MultiVariate Statistical Package for Windows, version 3.1. Kovach Computing Services, Pentraeth, Wales, U.K.
- Lampkin N.** 1990. *Organic Farming*. Farming Press Books, 701 pp.
- Legendre, P., Legendre, L.** 1998. *Numerical Ecology*. Elsevier Science B.V., Amsterdam, 853 pp.
- Leonard, C.** 2001. Soil properties that influence grassland production: their identification, production-indicator potentials and explanatory mechanisms. Unpublished PhD thesis, University of Limerick.
- Lin, Q., Brookes P.C.** 1999. Arginine ammonification as a method to estimate soil microbial biomass and microbial community structure. *Soil Biology and Biochemistry* 31, 1985-1998.
- Loveland, P., Webb, J.** 2003. Is there a critical level of organic matter in the agricultural soils of temperate regions: a review. *Soil and Tillage Research* 70, 1-18.
- Nannipieri, P., Grego, S., Ceccanti, B.** 1990. Ecological Significance of the Biological Activity in Soil. In: Bollag J.-M., Stotzky G. (Eds), *Soil Biochemistry*, Volume 6. Marcel Dekker, Inc. NY pp. 293-355
- Newton, J.** 1993. *Organic Grassland*. Chalcombe Publications, 128 pp.

- Norušis, M. J.** 1992. SPSS / PC for Windows: Base System User's Guide, release 6.0. Chicago IL SPSS Inc.
- O'Sullivan, A.M.** 1968. The lowland grasslands (Molinio-Arrhenatheretea) of County Limerick. An Foras Talúntais, Dublin. 57 pp.
- Palmer, M.** 2000 Principle components analysis  
[www.okstate.edu/artsci/botany/ordinate/PCA.htm](http://www.okstate.edu/artsci/botany/ordinate/PCA.htm)
- Richards, B.N.** 1987. The Microbiology of Terrestrial Ecosystems. Longman Scientific and Technical, U.K., 399 pp.
- Rowell, D.L.** 1994. Soil Science Methods and Applications. Longman Scientific and Technical, U.K., 350 pp.
- Sparling, G.P.** 1997. Soil Microbial Biomass Activity and Nutrient Cycling as Indicators of Soil Health. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R.(Eds.) Biological Indicators of Soil Health. CAB International, pp. 97-119.
- SPSS** 1999. SPSS for Windows, Release 10.0.5 (November 1999). Network version SPSS Inc., 1989-1999.
- Whitehead, D.C.** 1995. Grassland Nitrogen. CAB International 397.