Repeated-measure analysis of the temporal nitrous oxide emissions from the multi-species mixtures

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Declaration

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Repeated measures analysis was applied to analyse the nitrous oxide (N_2O) emission observed from an experiment that consisted of controlled agricultural plots with evenly distributed mixtures of species from three different functional groups: two grasses (L. perenne and P. pratense), two legumes(T. repens and T. pratense) and two herbs (C. intybus and P. lanceolata). Observations were preprocessed to create repeated measures data, based on seasons and the time of fertiliser application. The concepts of mixed-models and diversity-interaction (DI) models were combined to develop statistical models to predict the seasonal variations in the diversity effects in the multi-species mixtures. The combined statistical models were extended to include the effect of multiple levels of fertiliser application as well. Effect of reduced fertiliser application is found to be a dominant factor for seasonal variations in the N_2O emission. Model which considered diversity effects and the effect of reduced fertiliser application was able account for 86.2% variation in the seasonal N_2O emission. Multiple DI models were fit on on the N_2O emissions observed immediately after fertiliser application. Comparison of multiple DI models shows that significant identity effects were observed consistently after each fertiliser application, whereas a significant diversity interaction effect was observed only once between legumes and herbs.

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Chapter 1

Introduction

1.1 Background

Agriculture, forestry, and other land use (AFOLU) contributes to 21% of the total greenhouse gas (GHG) emissions and nitrous oxide (N_2O) is one of them. Around 75% of the N_2O emissions are from the agricultural activities. Manure applications (22%) and synthetic fertilisers (18%) to grasslands are the main sources of N_2O emissions (Velthof and Rietra (2018)). According to the IPCC (2019) report, unprecedented rates of increase of the concentration of the major greenhouse gases (carbon dioxide, methane, and nitrous oxide) have been observed, compared to at least the last 800,000 years. N_2O emission is one of the major concerns in the agricultural industry, with 256 times higher global warming potent, compared to CO_2. Therefore, factors responsible for the N_2O emissions should be critically analysed to make necessary changes required in current agricultural practices to curtail further N_2O emissions.

Emission of N_2O from an agricultural land is affected by a wide variety of biological and environmental factors, controlling many of those factors would be a tedious task. However, some of the major factors that influence the N_2O emission can be controlled. Synthetic nitrogen (N) based fertiliser application directly affects the amount of N_2O emission (Harty et al. (2016)) from a grassland. Hence, one of the ways to reduce N_2O emission from agricultural land would be the efficient use of the N fertiliser. Just reducing the amount of fertiliser application may not be ideal, as it would compromise the yield. Mixture of multiple species based on the ability of nitrogen-fixing ability can reduce N_2O emissions as well. The N use efficiency (NUE) can be improved with mixtures of grasses, legumes, and herbs (Suter et al. (2015)). Moreover, the mixtures also provide other benefits like increased yield, greater yield, stability, weed suspension, and improved animal performance (Cummins et al. (2021)). Therefore, mixture of the right compositions of species could be the answer for the sustainable intensification of agriculture with reduced emission of N_2O.

Amount of fertiliser application and composition of species in a grassland can be controlled with the possibility of minimising the N_2O emission. However, details about the appropriate amount of fertiliser to be applied or the right proportion of species that can minimises N_2O emission under various climatic and biological conditions is still unknown. To answer these questions, it is necessary to understand the quantitative dynamics of various factors like the effect of of fertiliser application, species diversity effects, seasonal effects, and other factors which could describe the the observed N_2O flux. Recent findings by Cummins et al. (2021) shows that annual N_2O emission can be effectively reduced up to 63%, from the plots with multi-species mixtures compared to the monoculture plots, with the same amount of dry matter yield. It reports the analysis from the the annual N_2O flux, and this analysis can be extended to consider the dynamic changes in the effects over the time of the experiment. Temporal analysis of the factors, which affect the N_2O emissions could be useful to explore further options, like identifying the right time to apply fertiliser, or other treatments to achieving the reductions in N_2O emissions.

1.2 Motivation

The motivation behind the analysis of N_2O emission lies under the fact that N_2O is expected to be the dominant ozone depleting agent and urgent actions need to be taken to limit its emission. Mixed species compositions can provide a variety advantages in terms of improved yield, improved stability, and reduced N_2O emissions. In-depth understanding of the factors which affect N cycle in the mixed species environment is necessary to decide the urgent actions that can reduce N_2O emission without affecting the yield. This dissertation extends the analysis reported in Cummins et al. (2021), to analyse the temporal effects on N_2O emissions. The thesis is an exploratory analysis that reports the predicted strength of the various effects that causes variations N_2O emissions with time. The goals of the thesis are listed as follows,

1. Analyse variations in the identity effects of different functional groups on N_2O emission over multiple applications of fertiliser.

2. Analyse variations in the interactions between functional groups on N_2O emission over multiple applications of the fertiliser.

3. Analyse seasonal variations in the identity effects of different functional groups, on N_2O emission.

4. Analyse seasonal variations in the interactions between functional groups on N_2O emission.

1.3 Overview

The thesis structure is explained in this section. To start with, Chapter 1 starts with a brief background about the work. The motivation and goals of the thesis are presented after explaining the background. Finally, overview of the the thesis is presented. Next, in Chapter 2, recent research works in the related field of study and their contributions are reviewed. This chapter is structured to cover the research works related to the mixture problem, analysis of N_2O emission, species interactions, repeated-measures analysis and concluded with the summary of the literature reviewed. Chapter 3 provides technical details about the experimental design principles, modelling approaches, and evaluation methods used in various stages of the thesis. Implementations of the models are provided in Chapter 4. Which covers the data preprocessing steps for different types of models used in the thesis, and details about the implementation in R. Results from the models implemented sin Chapter 4 are summarised in Chapter 5. Evaluations of the models and the coefficients estimated from them were reported and analysed. Finally, the conclusions from this thesis are presented in Chapter 6, it also covers details about possible directions for the future works based on this thesis.

Chapter 2

Literature Review

Current productions from grassland-based agriculture are mainly based on pure grass monoculture. Pure grass monoculture produces a high yield but require a large amount of synthetic nitrogen(N) based fertilisers, like calcium ammonium nitrate (CAN). Extensive usage of N based fertiliser results in a substantial amount of N loss in the form of nitrous oxide (N_2O) (Harty et al. (2016)), which is a dominant greenhouse gas (GHG) responsible for ozone layer depletion in 21^{st} century(Ravishankara et al. (2009)). Therefore, in recent times, research works on studying the N_2O emission and analysing the dependent factors have gained traction. These research works are trying to explore the various options to minimise the N_2O emission from the grasslands.

This chapter explores some of the recent research works that employed statistical methods to analyse the emission of N_2O and its effect on the environment. To start with mixture problem and relevant methods to solve it are being discussed. The next section emphasises recent findings on the N_2O emissions. Various ways of analysing the species interactions are being explored in the later section. The final section focuses on the methodologies involved in the repeated-measure analysis.

2.1 Mixture Problem

The problems related to creating mixtures with various combinations of proportions of pure components on the response of the system are known as mixture problems. Mixtures are created to facilitate the analysis of the effect of each individual component or combination of components. In the experiments which involve the analysis of mixtures, the response variable depends on the proportion of the components in a mixture and not on the total amount of the components. For example, the study of the properties of an alloy, like the tensile strength and wear resistance, depends on the proportion of different metals in the alloy. Various methods were proposed to create combinations of points in the factor space to design a mixture of components (Cornell (1973)). Two methods to solve the mixture problem, which is relevant to this experiment are being reviewed in this section.

Scheffé (1958) was the first research work to provide a strategic design to solve the mixture problem and simplex-lattice design was introduced in it. In this design, a $\{q, m\}lattice$ will be created, which contains $m+1$ equally spaced values from $0, 1/m, 2/m, ..., 1$ and all combinations of these proportions for each of the q components. Image 2.1 shows the visualisation of simplex-lattice created for different values of q and m.

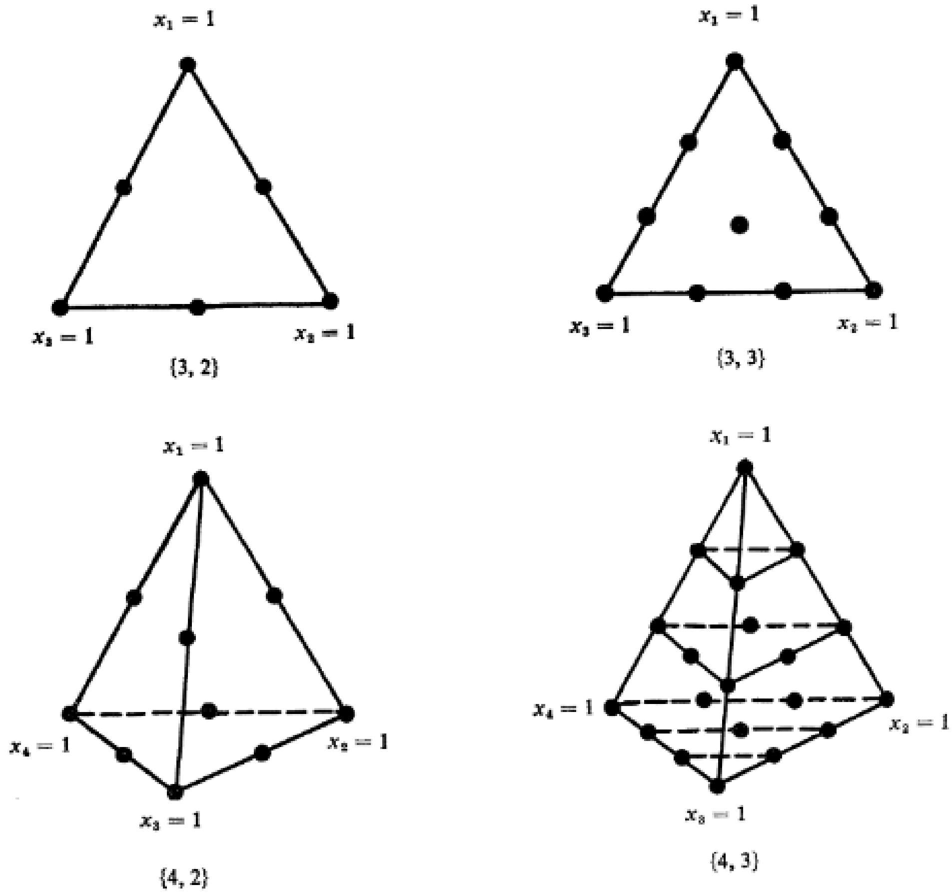

Figure 2.1: Examples of simplex-lattice in different factor spaces as illustrated in Scheffé (1958)

Simplex-lattice design is a simple solution for solving the mixture problem, however, the proportions of the components are not equal in all mixtures, especially when there are more than 3 components. This asymmetry makes it difficult to interpret the strength of the interaction between more than two components.

The simplex-centroid design was introduced in Scheffe (1963), to fix the problem of asymmetry in the simplex-lattice design. In this design, for q components, q pure components are created with proportions $(1, 0, ..., 0), (0, 1, ..., 0), ..., (0, 0, ..., 1)$, $\binom{q}{2}$ permutations of $(1/2, 1/2, ..., 0)$ and so on, and a point $(1/q, 1/q, ..., 1/q)$. Symmetric mixture compositions are provided by simplex-centroid design to analyse all possible orders of interaction that could affect the response of the mixtures. More detailed discussion about modelling the strength of interactions in the mixtures created by simplex-centroid method can be found in 3.1.1.

However, the simplex-centroid method has some limitations. The estimates of the response variable vary greatly over the simplex when higher order polynomials are included. The simplex-centroid design allows creating mixtures with process variables, for example, considering factors like breed or age of a cattle for creating mixtures of cattle feed. The design assumes that the variation in the response is homogeneous. However, there is a possibility of error at two levels when the process variables are considered along with the compositions of mixture components. One at the subject level and another at the process variable level, for example, considering the cattle feed mixture analysis example, there could be one error level for each cattle and another for the subgroups based on age or breed of the cattle.

A wide range of research works, which aims to study the effect of species mixtures in a grassland used simplex-centroid(simplex) design. The research by Kirwan et al. (2007) analyses the possible effect of species mixtures and other factors like evenness and richness in the community, on the yield. As part of the experiment, mixtures of 4 species, two grass and two legumes were created for 28 sites spread across 17 countries. Total 30 plots were created with various proportions of each species using the simplex method. The research work by Suter et al. (2015) demonstrates the application of simplex design to analyse the amount of N_2O emission from the grassland mixture of grass and legume. It aims to compare the emissions from monoculture plots and grass-legume mixtures with different proportions of 4 species in the experiment. The next section reviews some of the recent research works related to N_2O emission from agricultural plots.

2.2 N_2O emission

Ozone depletion potential (ODP) is a measure that quantifies the relative contribution of various ozone-depleting substances (ODSs). Comparing the ODP-weighted anthropogenic emission of N_2O with other ODSs shows that, N_2O is currently the largest ozone-depleting emission. Further analysis to predict the future emission of N_2O and its long term effect on ozone concentrations, confirms the necessity to limit the N_2O emissions in the near future (Ravishankara et al. (2009)).

Emission of N_2O is not regulated in the AFOLU economic sector, hence, it is a common practice to use excessive fertiliser to obtain high yields of crops. The excessive usage of N based fertiliser results in higher amounts of N_2O emission. Fertiliser application is not the only factor responsible for the emission of N_2O, various factors, like weather conditions, soil texture, soil minerals with N, etc also influence the emission. However, with appropriate changes in conventional practices in agriculture, there is a good possibility to reduce N_2O emission from AFOLU economic sector. For example, changing from the synthetic N based fertiliser to urea-based fertilisers (Harty et al. (2016)), or managing fertiliser application in the presence of grazing animals (Maire et al. (2020)), or using grass-legume mixtures to support N fixing in soil (Suter et al. (2015)), shows some of the possible options.

A two-year experiment was conducted over six permanent pasture sites in Ireland to show the possibility of limiting N_2O emission on average by 80% by using urea based fertilizer formulation instead of the synthetic N based fertiliser like CAN (Harty et al. (2016)). Minimum N_2O emission was observed in the sites where urea-based fertiliser was applied along with nitrification inhibitors (NIs), like *Dicyandiamide* (DCD) and urease inhibitors (UIs), like *N-(n-butyl) thiophosphoric triamide* (NBPT). this experiment employed a novel approach of using statistical methods like mixed models, discussed in detail in section 2.4, to analyse N_2O emissions from different groups of fertiliser and inhibitor applications.

A well-designed experiment was conducted for 3 years in sites across different parts of Europe, by Suter et al. (2015), to statistically analyse the effect of the interaction between grass and legume species on the amount of N_2O emission. This novel approach which aims to achieve the sustainable intensification of agriculture, which involves improving the productivity from the land but at the same time minimising its environmental effects. This experiment confirms the possibility to reduce N_2O emissions from soil, using the symbiotic N_2 fixing property of some of the legume species like *Trifolium pratense* L. and *Trifolium repense* L. Legumes are capable of fixing naturally available in N_2 into the soil in the form in N based minerals, ranging from 100 to 380 kg ha^{-1} yr^{-1}. These minerals are available to be used by grasses in the grass-legume mixture, hence reducing the fertiliser requirement and in turn substantially reducing N_2O emission. Statistical methods were used in the experiment to model the effect of species interactions. More details about the statistical approaches for species interactions are discussed in the section 2.3.

2.3 Interactions in species mixture

In a given biological environment, different species interact at different levels and in complex ways. Especially in a system with more than two species, it is difficult to understand the pattern of interactions among these species. Hence, many research works apply advanced statistical techniques to model the patterns of interactions between species in a given biological system. To get accurate results from statistical analysis, experiments need to be carefully designed to minimize the variations in the outcomes based on factors that can not be controlled and strategic models are also necessary to correctly interpret the effect of species interactions.

The simplex design can be used to decide the proportions of the species in various agricultural fields to analyse the effect of species and their interactions on N_2O emissions. The DI modelling framework can be applied effectively to model the ecosystem function using the data collected from the experiment which involves species mixtures designed using the simplex method. Various effects, like identity effect, species interaction effect, functional group interaction effects, and etc, on the outcome of ecosystem functions, like yield or N_2O emissions (Kirwan et al. (2009)), can be modelled using the DI modelling framework.

In a species-rich ecosystem, two species may interact and affect the outcome of the ecosystem positively, negatively, or neutrally. Modelling the species interactions gets complex as the number of species in the ecosystem increases. Brophy et al. (2017a) proposes methods to minimize the complexities associated with understating the contribution of species interaction to the ecosystem outcome. It extends the DI modelling framework (Kirwan et al. (2009)) by including fixed effects and random effects to understand the effects of diversity on the outcome of the ecosystem.

The application of the DI modelling framework was demonstrated in an experiment (Brophy et al. (2017b)) that was conducted in 31 sites across Europe and Canada for 3 years to identify the persistence of yield benefit across years, sites, and mixture communities. It also aims to identify the contribution of functional trait levels to the diversity effect. Four different species representing two functional traits, nitrogen fixing and temporal development, were used in this experimental design to increase complementarity in resource usage. The yield benefit across mixture communities was modelled using DI modelling framework to interpret the identity effects and species interaction effects.

Recent research by Karakoç et al. (2020) demonstrated the analysis of the time-varying species interaction effect. This research work demonstrates the use of empirical dynamic modelling (EDM) methods (Sugihara et al. (2020)) to quantify the time-varying strength of species interactions. EDM is a non-parametric framework that uses the mathematical theory of reconstructing attractor manifolds using time series data. Non linear dynamic systems can be effectively analysed using EDM, however, it is not designed specifically for analysing species interactions and the results from EDM have a considerable number of limitations. DI modelling is a framework specifically designed for understanding the various effects of species mixtures on the outcome of the ecosystem. However, it does not provide a way to analyse temporal changes in the strengths of species interactions and the changes in the ecosystem along the time. Therefore, developing temporal diversity interaction models, which combine the concepts of the DI modelling framework and repeated measure analysis, could be helpful in understanding the temporal dynamic effects of species mixtures on the functional response. Various approaches to analyse the temporal data using repeated measure analysis are reviewed in the next section.

2.4 Repeated-measures analysis

To analyse the temporal effect of species mixtures on the outcome of the ecosystem, one of the possible options is to consider the temporal outcome of the ecosystem as repeated measure data recorded from the individual plot(subject). In repeated measure data, multiple readings are taken for the response from the same subject. For example, N_2O emissions readings taken from each season from the same plot. Since the repeated measures are not independent responses, as they are taken from the same subject, analysis approaches should account for the covariance across multiple measurements. Methods of analysing repeated measure data can be applied effectively to the data which contains multiple readings of different subjects over time. A variety of methods are available to analyse repeated measure data and each method has different assumptions associated with it. Therefore, to choose the right modelling methods suitable for the experiment, it is important to critically review the suitable methods before using them. This section reviews some of the commonly used repeated measure analysis methods (Maurissen and Vidmar (2017)).

2.4.1 Multiple Comparisons

In experiments that involve the analysis of relationships among multiple levels of independent variables, like analysing the outcome of the ecosystem based on multiple applications of the fertiliser, multiple comparison tests can be the simplest option. Various tests and contrasts are available to compare multiple measurements like the least significant difference test, honestly significant difference test, Newman-Keuls test, Duncan test, Scheffé's method. However, each test has specific use cases, assumptions, and requirements associated with it (Zar (2009)).

Although there are multiple tests to compare multiple measurements, these methods have limitations associated with them. Overall, the Type I error rate across multiple comparisons increases with the increase in the number of comparisons, which is technically called a multiplicity problem. Inflation in the error rate can be minimised using some mathematical methods. However, it is important to consider the possibility of multiplicity problems during the design of the experiment and variable selection for the analysis.

2.4.2 RM-ANOVA

Repeated measure analysis of variance (RM-ANOVA) can be used to analyse a finite number of repeated measurements and it considers the correlation within the subject(Hayat and Hedlin (2012)). This is an old method to analyse repeated measures, but it can be a powerful method to identify an effect if it exists when the underlying assumptions are satisfied. The requirement of satisfying the assumptions poses a number of limitations for using RM-ANOVA.

One of the limitations in RM-ANOVA is the requirement of a balanced design. Balanced design means repeated measures should be collected at the same time or conditions for each subject, which belongs to a finite number of categories being observed. If a subject has one or more missing measurements, that subject will be entirely dropped from the analysis. This could lead to biased results as there could be a possibility of a significant effect among the subjects for which the observations are missing. Which in turn results in misinterpretation of the observations with very less or no real world impact. Another limitation of RM-ANOVA is that the results are not easily interpretable as they do not provide parameter estimates like the results from linear regression. Mixed-models overcome these limitations and it would be a better option over RM-ANOVA to model repeated measure data.

2.4.3 Mixed-models

Mixed-models (also known as multilevel models, hierarchical models) are one of the useful modelling frameworks for data that contains multiple subgroups. For example, analysing the response to treatment from patients of different age groups. This modelling technique provides a way to model the variations within the group and variations across the groups. Technically, mixed-models are a generalisation of linear regression with varying slope and intercepts by group (Gelman and Hill (2006)). Population mean is inferred using hypothesis testing or confidence intervals. The covariance structure of the data needs to be accounted for the right interpretation of the model.

One of the main advantages of using mixed-models is that it provides a robust and flexible way to analyse the repeated measured data with limited assumptions to be satisfied. Missing data does not affect the overall analysis using Mixed-models as it will use all available data. However, missing data itself may have a potentially important pattern, which may cause bias in the result. Therefore, the details about the missing data need to be reported while applying the mixed-models.

2.5 Summary

Current agricultural practices are directly impacting the rise in N_2O emissions in recent times. Various factors, like fertiliser application, species interactions, weather conditions, mineral levels in soil, microbial activities, etc, affect the N_2O emission from agricultural land. Temporal analysis of the N_2O emission based on the effect of the above-mentioned factors can be achieved by using the repeated measures analysis techniques. Analysing a repeated measure data can be a challenging task, as there are various methods available and each method has its own limitations and assumptions to be satisfied. Various aspects of the analysis methods should be carefully analysed to identify the best suitable analysis method for the data and the hypothesis being analysed.

Chapter 3

Design and Methodologies

Statistical analysis and modelling involves applying a wide variety of techniques and methodologies at various stages. This section provides a detailed explanation about the methodologies involved in each stage of the analysis. First, the methods used for designing the experimental are discussed. Experiment design emphasises on creating mixtures of species using the simplex method. Details about the process involved in the calculation of the response variable, N_2O flux, is covered as part of the experimental design section. Second, DI modelling framework is explained, which creates the main foundation for the models analysed in the implementation (add reference) chapter. Mixed-models and multiple comparison techniques are also discussed as part of the modelling methods section. At last, the statistical tests employed for the evaluation of goodness of fit and hypothesis testing are discussed.

3.1 Experimental Design

The experiment was designed to capture N_2O emission from the mixtures of six species belonging to three different functional groups: two grasses (L. perenne and P. pratense), two legumes(T. repens and T. pratense) and two herbs (C. intybus and P. lanceolata). Input and output of the experiment are the two major factors, which need to designed carefully to produce data which can be used for statistical analysis. The measurement of input variables of the experiment should be carefully designed to satisfy the assumptions of the statistical models, which will be used for analysis and to avoid biases in the measured data. The output or response variable of the experiment is N_2O flux. N_2O flux was calculated by analysing concentration N_2O emitted from each plot using the gas chromatography technique. Detailed discussion about the N_2O flux calculation is discussed later in this section. Upcoming subsections first explain about the simplex design, which was used to design the plots with different proportions of species, next describe the experiment setup , and later methods involved in the measurement of N_2O emission was discussed.

3.1.1 Simplex design

Simplex design is used to design the mixture experiments to get a uniformly spread distribution of the proportions of all components involved in the experiment (Scheffe (1963)). For q components, this design would generate permutations of q pure components, $(q/2)$ binary mixtures, $(q/3)$ tertiary mixtures and so on with equal proportions. Simplex method provides a way to combine process variables also while designing a mixture, for example, in a mixture design based on analysing the proportions of cattle feed, it is possible to introduce variation based on the age and breed of the cattle (Cornell (1973)).

Mixtures created with the simplex method can be used effectively to model polynomial regression models to analyse the strength of variation in the response based on the mixture effects of components. For example, consider a mixture created with q components, which produce points $x_1, x_2, ..., x_q$ in a simplex. A polynomial regression model can be fit for these points (representing the proportions of each component) as follows,

$$Y = \sum_{1<=i<=q} \beta_i x_i + \sum_{1<=i<j<=q} \beta_{ij} x_i x_j + \sum_{1<=i<j<k<=q} \beta_{ijk} x_i x_j x_k + ... \tag{3.1}$$

In above equation 3.1, β_i can be interpreted as the strength of an individual component, β_{ij} can be interpreted as the strength of interactions among the components i and j, and so on. One of the main goals of this experiment is to model the effects of individual species and the interactions between different species in the mixture. Therefore, the simplex method can be applied to design plots, such that the strength of the various effects in the mixture can be analysed with a regression model.

3.1.2 The Experiment

Using the simplex design method, 20 communities of species mixtures were created with symmetrically varied proportions of functional groups. Distribution of the three functional groups: grass, legume, and herb, in the communities created for the experiment is shown in the figure 3.1. Total 43 plots were created for the field experiment with 1-4 repetitions of each community. The field experiment was conducted for a year, March-2018 to March-2019, in Teagasc, Johnstown Castle, Co. Wexford, Ireland $52°18'27$. Tabulated summary of the proportions of species in each community and their repetitions is provided in (**Appendix A**).

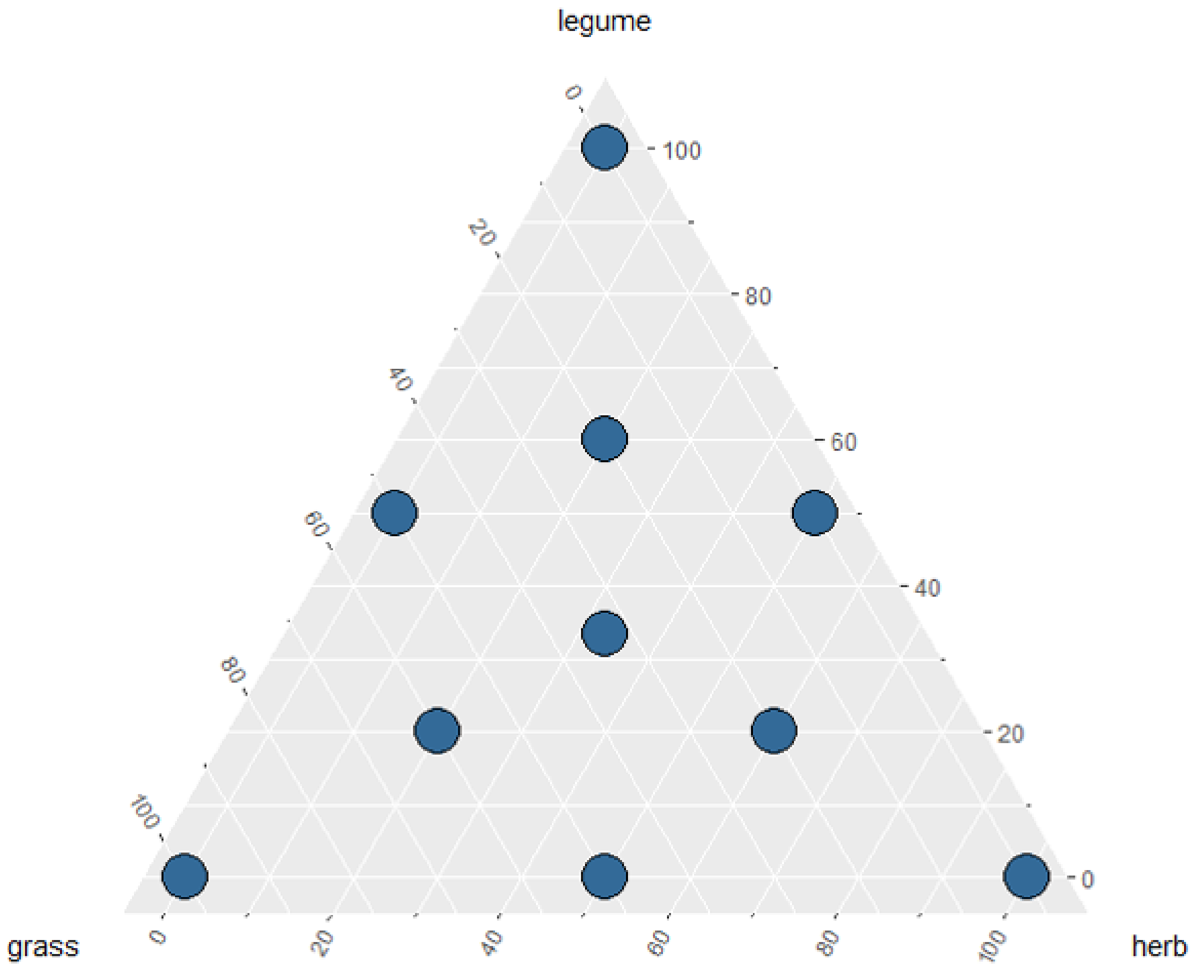

Figure 3.1: Distribution of the proportions of the functional groups, grass, legume, and herb, created using simplex design

Fertiliser was applied 5 times with varying time intervals, to the plots in the first six months of the experiment, March-2018 to September-2018. First 19 communities (1-19) are the experimental sites, which were applied with reduced amount of fertiliser at the rate of 150 kg N ha^{-1} $year^{-1}$. Community 20 is the control site, which simulated the regular agricultural practice of fertiliser application on grass monocultures. Fertiliser was applied at the rate of 300 kg N ha^{-1} $year^{-1}$. Specific details of the fertiliser application is summarised in table (**Appendix B**).

Emissions of N_2O was measured with varying intervals throughout the time of the experiment from March-2018 to March-2019. To capture the N_2O emissions induced by fertiliser application, air samples were collected more frequently, four days a week for two weeks, immediately after the fertiliser application. Air samples were collected less frequently, two days a week for the next two weeks and one day a week, until the next fertiliser application. High resolution sampling strategy was used for the first six months of the experiment (March-2018 to September-2018). In the last six months of the experiment, low N_2O emissions were expected because of low soil temperature and no N based fertiliser application. Therefore, a less intensive sampling approach was employed and emission was measured at a frequency of once per month.

3.1.3 Nitrous oxide flux measurement

Static chamber methodology was used to measure nitrous oxide emission with each plot consisting of one chamber. While sampling, 10 ml air was collected using a syringe and preevacuated glass vials, with necessary precautions to avoid contamination of the collected air. Measurements which reflect the average hourly flux of the day were calculated using the collected gas samples. Nitrous oxide concentration was measured using a gas chromatograph(GC, Varian CP 3800 GC, Varian, USA) (Cummins et al. (2021)). N_2O flux (response variable) was calculated using linear regression of the increasing concentration of nitrous oxide over multiple samples collected over a short time (3 samples with a 20 minute gap), for each of the gas samples collected from the chambers in each plot.

3.2 Modelling methods

Statistical modelling provides a wide variety of methods to data consisting of various structures and to analyse different kinds of hypotheses. The data collected in the experiment, explained in the previous section 3.1, is a temporal observation of N_2O flux from the agricultural plots with different mixtures of species. Concepts from multiple approaches, like DI modeling, multiple comparisons, and mixed models, were combined to build models to analyse the changes in the dynamics of various effects in mixtures with multiple observations of response. This section explains the methods and their adoption in this analysis.

3.2.1 Diversity-interaction modeling

DI modeling (Kirwan et al. (2009)) framework provides a flexible way to evaluate various kinds of hypotheses related to communities consisting of multiple species. Response of the community y is modelled as a function of the proportion of individual species P_i, identity effect(ID) and their combinations P_iP_j, diversity effects(DE) in the DI model. Equation 3.2 formulates the general form of the DI model and equation 3.3 segregates the terms representing the identity effects and interaction effects.

$$y = \sum_{1 \leq i \leq s} \beta_i P_i + \sum_{1 \leq i < j \leq s} \delta_{ij} P_i P_j + \varepsilon \tag{3.2}$$

$$\begin{aligned} \text{ID} &= \sum_{1 \leq i \leq s} \beta_i P_i \\ \text{DE} &= \sum_{1 \leq i < j \leq s} \delta_{ij} P_i P_j \end{aligned} \tag{3.3}$$

Where s is the number of species in the community, β_i indicates the strength of the effect of individual species i on the response of the mixture community, and δ_{ij} is the strength of the interaction between species i and j. Error term in the model is represented by $\varepsilon \sim \mathcal{N}(0, \sigma^2)$.

Structural details like blocks, densities, or treatments can also be modelled using the DI modelling framework. It also provides a way to model various patterns of interactions, like even interactions, additive interactions, pairwise interactions between all species, and functional group interactions. The species which are functionally redundant can be grouped together to analyse the identity effects and interaction effects between different functional groups. This analysis will be focusing on the functional group effects as the interaction model would become complex with 22 coefficients, if all six species are considered.

Observe that the DI model presented above can be used, when only one set of values of the response was collected from each of the plots. Data generated from the above explained experiment 3.1 has multiple observations for each plot. The changes in the strengths of various effects in the mixture across multiple measurements can be analysed in two possible ways. First, by comparing the coefficients of DI model fit for different sets of observations. Second, by fitting DI models in the mixed-model structure by considering various effects in the mixture as random and fixed effects.

3.2.2 Mixed-models

A simple linear regression model for the response variable Y and the predictor variable X is represented as follows,

$$Y = \alpha_0 + \beta_0 * X + \varepsilon \tag{3.4}$$

where, α_0 is the intercept of the model, β_0 is the coefficient of the model, and $\varepsilon \sim \mathcal{N}(0, \sigma^2)$ is the error term in the model. Linear regression is ideal for fitting data which consists of predictor variables which are correlated with the response variable. However, linear regression will not be able to fit the data correctly when it contains groups (or levels, categories, hierarchies, repeated measures, etc). Groups in the data may exist based on the specific properties of the subjects of the experiment, like patients belonging to different age groups would respond differently to the same treatment, or it could be based on the design of the experiment, like multiple measurements of the patients response to the same treatment. In such cases, the observed responses could be dependent on the grouping factor, which violates the assumption of independent response variables for applying linear regression.

Mixed-models are the extension of the linear regression to accommodate the group level variations in the data. Coefficients of the linear regression are maintained in the mixed-models as a fixed effect, which models the covariance of parameters X, across all groups. Error term ε in linear regression is expanded to model the group level covariance of parameters Z within each group. Thus, the simple mixed-model with $j = 1, ..., J$ groups can be expressed as,

$$Y = \alpha_0 + \beta_0 X + \sum_{1 \le j \le J} (\alpha_j + \delta_j Z) + \eta \tag{3.5}$$

Where α_j represents the variation in group j (group level estimate of intercept) around fixed effect intercept α_0. Random effect coefficients of the group level variable Z, are represented by δ_j, which captures the random variations in the response in each group. The error term in the model η is expected to be normally distributed with mean 0, $\eta \sim \mathcal{N}(0, \sigma^2)$. The parameter X is considered only as a fixed effect in equation 3.5, if it needs to be considered as a random effect as well, then additional term $\beta_j X$ can be added to the above equation 3.5. Where β_j represents the variations, around β_0, in the response caused by parameter X, in each group.

Repeated measure data can be seen as a data containing a group of subjects for each measurement level. Therefore, repeated measures can be modelled using mixed-model. The experiment explained in the previous section 3.1 produces a repeated measure data. By combining the concepts of DI models and mixed-models, identity and interaction effects can be considered as random or fixed effects to fit various models for the repeated measure data. For example, consider a mixed-model with identity effects of the species, which are modelled as fixed and random effects with no intercept. By combining equation 3.2 and equation 3.6

$$Y_t = \text{ID}_{\text{fix}} + \text{ID}_{\text{ran}} + \eta \quad , \text{for } t = 1, ..., T$$

$$\text{ID}_{\text{fix}} = \sum_{1 \le i \le s} \beta_{i0} P_i \tag{3.6}$$
$$\text{ID}_{\text{ran}} = \sum_{1 \le i \le s} \beta_{it} P_i$$

Where Y_t represents a response for t^{th} measurement (among T measurements). The fixed effect in the model is represented by $\sum_{1 \le i \le s} \beta_{i0} P_i$, with β_{i0} representing the strength of fixed identity effect (ID_{fix}) of each species i. The Random effect in the model is encoded in $\sum_{1 \le i \le s} \beta_{it} P_i$, with β_{it} representing the strength of random identity effect (ID_{ran}) for species i and measurement t.

Different implementations of mixed models and DI models were analysed in this project. Implementation details of various models with different combinations of mixture effects and mixed model effects, which were analysed in this project can be found in chapter 4. On top of the effects presented in the equation 3.6, random diversity effect (DE_{ran}) and the random fertiliser application level effect (FL_{ran}). Random diversity interaction effect includes all pairwise interactions between species from different functional groups. In the mixed model DE_{ran} is encoded as shown in the equation 3.7 below, notice that the parameter δ_{ijt} is the coefficient representing the strength of the diversity interaction effect between the species i and species j in the for t^{th} measurement.

$$\text{DE}_{\text{ran}} = \sum_{1 \le i < j \le s} \delta_{ijt} P_i P_j \tag{3.7}$$

Random fertiliser level effect, FL_{ran}, is the effect of using reduced fertiliser level on the seasonal observation of the response. This effect is encoded in a single variable, therefore only one coefficient is enough to estimate this strength of this effect. The effect, FL_{ran} is encoded in the mixed model as shown in the equation 3.8, where, F_level is a variable indicating if a plot was applied with reduced fertiliser or not. And ω_t is the estimate of the effect of reduced fertiliser level for the t^{th} measurement.

$$\text{FL}_{\text{ran}} = \omega_t F_{level} \tag{3.8}$$

3.3 Evaluation Methods

Using statistical methods, a hypothesis about the data can be tested using a wide variety of methods based on the context of the analysis. In this section, two relevant methods of hypothesis testing are discussed. The first one is using the statistical significance of the parameters involved in the model. Statistical significance evaluation uses p-value, which indicates the probability of occurrence of an effect, assumed by the hypothesis, given the null hypothesis. More detailed discussion about statistical significance is presented in Appendix C. Second way to test the hypothesis is by evaluating the "goodness of fit" of the statistical model, which was fit based on the assumptions of the hypothesis. Goodness of fit of the model can be evaluated using residual analysis of the model using tests like deviance test, AIC and BIC test, pseudo R^2 test, etc.

Mixed-models were compared based on a goodness of fit test to identify the factors that provide a best fitted model. Implementation chapter 4 explains the six mixed-model, model that encodes the majority of variation in the response variable was chosen based on the results of the goodness of fit test like AIC, BIC, deviance and pseudo R^2. The coefficients of multiple DI models, which were fitted based on the time of fertiliser applications, were check checked for statistical significance to identify if any significant diversity effects were observed in the experiment.

3.3.1 AIC and BIC

Akaike information criteria (AIC) and Bayesian information criteria (BIC) provide relative measures for model selection, based on the estimation of error in prediction. Likelihood of the model can be increased by adding more predictor variables, which would result in over fitting of the model. To overcome this problem, both AIC and BIC add a penalty term based on the number of predictor variables.

AIC rewards the goodness of fit based on the likelihood function, which discourages under fit, and penalises for the larger number of parameters, which ensures a higher penalty for over fitting. Mathematically, AIC can be expressed as shown in the equation 3.9

$$AIC = 2k - 2\ln \hat{L} \tag{3.9}$$

where k is the number of predictor variables and $\hat{L}$ is the maximum value provided by the likelihood function for the model.

Equation for BIC 3.10 is very similar to AIC, except the penalty for the number of predictor variables $log(n)$, which is relative to n, the number of samples, instead of a fixed value of 2 in AIC.

$$BIC = k\ln(n) - 2\ln \hat{L} \tag{3.10}$$

where k is the number of predictor variables, n is the total number of samples, and $\hat{L}$ is the maximum value provided by the likelihood function for the model.

Model with lower AIC or BIC is considered as the best model based on requirements of the analysis. Some researchers argue that BIC should be considered instead of AIC for the selection of "true model" which indicates the process which generated the data. However, other researchers argue that AIC may have negligible differences compared to BIC, depending on the ratio of sample size n and the number of parameters k in the model.

3.3.2 Residual deviance

Analysis of the goodness of fit of the linear model, fitted with the ordinary least squares (OLS) method, uses the sum of squares of residuals (RSS). Generalisation of RSS for models, which fits the linear mixed-model using the maximum likelihood function, is called the residual deviance. Residual Deviance is a function of the log-likelihood of the fitted model and can be expressed as shown in the equation below,

$$D = -2 * LL(\textbf{Fitted Model}) \tag{3.11}$$

where D is the residual deviance, LL is the log- likelihood function for the fitted model.

Multiple models, based on different hypotheses, can be compared by changing the model parameters and keeping the response variable same. The Hypothesis corresponding to the lowest residual deviance can be considered as the best explanation of the data compared to other hypotheses, as the model corresponding to that hypothesis fits the data better than other models.

3.3.3 Pseudo R-squared

Comparison of the baseline model (which predicts the mean response) with the fitted model can be evaluated using R-squared statistic. For example, if a fitted model produces $R^2 = 0.9$, the input parameters included in the model account for 90% of the variation in the response variable. This method is very useful for hypothesis testing as it provides an easy way to compare and interpret the goodness of fit of the fitted model. Recent article Chicco et al. (2021) claims that R-squared value (coefficient of determination) can be more truthful than other evaluation methods, like MSE, RMSE, MAPE, and SMAPE, which are usually used for regression analysis.

Depending on the context of the compared models, R-squared values are defined separately. To get an intuition of the R-squared statistic, first consider the linear regression model fitted based on OLS. Following equation 3.12 shows the calculation of R-squared value for models fitted with OLS.

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}} = 1 - \frac{\sum_i (y_i - f_i)^2}{\sum_i (y_i - \bar{y})^2} \tag{3.12}$$

Where y_i is the observed response, f_i indicated predicted response from the fitted model, and $\bar{y}$ represents the mean of observed responses. Therefore, SS_{res} is nothing but the squared sum of residuals, or variance in the data from a model and SS_{tot} is the variance in the observed response variable.

For fitting the mixed-models, maximum likelihood estimation (MLE) is used and it consists of fixed effects and random effects. Thus, R-squared statistic for the predictions from mixed-models is derived as shown in equation 3.13. This is also referred as the pseudo R-squared statistic.

$$R^2 = \frac{\sigma_f^2 + \sigma_\alpha^2}{\sigma_f^2 + \sigma_\alpha^2 + \sigma_\varepsilon^2} \tag{3.13}$$

Where σ_f^2 is the variance of the fixed effect components, σ_α^2 is the variance of the random effect components, and σ_ε^2 is the variance of the observation.

Chapter 4

Implementation

Methodologies discussed in the previous chapters were employed to implement multiple mixed-model and DI models to understand the relationship between mixture composition, fertiliser, seasonal changes on the N_2O emission from the grassland. The models were implemented in R, version 4.1.0 (2021-05-18) in Windows 10 operating system. The mixed-models were implemented using an R package `lme4` (Bates et al. (2014)), and the models were evaluated with R package `lmerTest` (Kuznetsova et al. (2017)). For repeated measure analysis of N_2O emission on the application of fertiliser, `DIModels` R package, based on Kirwan et al. (2009), was used.

To start with, the data preprocessing steps are explained. Data preprocessing covers details about specific preprocessing steps applied to the raw data, which was collected from the experiment explained in section3.1. Next, mixed-model implementations and the hypotheses being tested are being explained. Later, the implementation of DI models is discussed. Finally, details about all models, which were implemented as part of this thesis, are summarised. The analysis of the results from the models discussed in this section is presented in the next section 5.

4.1 Data preprocessing

The raw data contains three tables. The first table has information about the different communities with various proportions of species in each of the 43 plots (Appendix A). Data from 42 plots were used for the analysis as the observations from one of the plots were missing. The second table contains the observations of N_2O flux at different intervals in each experimental plot, over a year from March 2018 to March 2019. The third table provides information about the application of nitrogen based fertiliser. Calcium ammonium nitrate (CAN) fertiliser was applied 5 times during the period of the experiment at varying intervals(Appendix B) in two different levels. These three tables were merged to produce a single data set, using R in *Tidy data* format. An additional column was added to the combined data set, which differentiates the two levels of fertiliser application. One of the goals of this work is to analyse the effect of reduction in the amount of fertiliser application, hence communities with a reduced amount of fertiliser application, 150 N, are encoded as 1 and the communities with a regular amount of fertiliser application, 300 N, are encoded as 0.

The data contains total 73 observations of N_2O flux at different intervals from each plot. Mixed models and DI models are complex in structure and having 73 repeated measures would make the model very complex and difficult to interpret. Therefore, to simplify the model, data is aggregated based on the time of the observation. There are multiple possibilities in which time segments can be considered for aggregation. Two aspects are considered in this analysis for aggregation of temporal N_2O flux observations. One is based on seasons and the other is based on fertiliser application. Seasonally aggregated data was used for mixed-model analysis and data aggregated based on fertiliser application was used for comparing the changes in the strengths of various effects in the species mixture using multiple DI models.

4.2 Mixed-model implementations

Mix-models were fit using `lmer` function defined in R package `lme4`. For fitting the coefficients, the maximum likelihood (ML) method was used instead of the default restricted maximum likelihood (REML). Options of the goodness of fit test for the model fit using ML are more compared to the goodness of fit test available for the model fit using REML. For fitting mixed-models response variable, N_2O flux, was scaled between 0-1, for achieving better convergence of the model.

4.2.1 Data aggregation based on seasons

The experiment was conducted in Teagasc, Johnstown Castle, Co. Wexford, Ireland therefore, observations are aggregated based on the seasonal patterns in Ireland. According to Met-Éireann (2021), Ireland has four seasons, Winter: from December to February, Spring: from March to May, Summer: from June to August and Autumn: from September to November. The observations of N_2O flux is aggregated by calculating total N_2O flux in each season for each plot. Total seasonal N_2O flux will be used as a dependent or response variable for the mixed-models defined in the next section. After aggregation, the data contains four repeated observations of aggregated N_2O flux for each plot. A visualisation of seasonal aggregated data of the plots separated based on the levels of fertiliser application is shown in image 4.1.

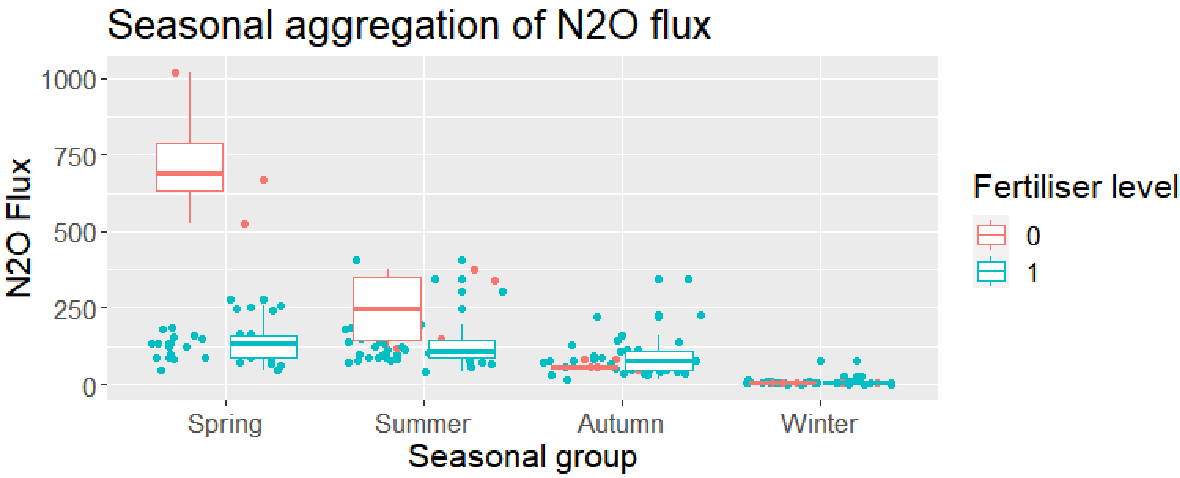

Figure 4.1: Total N_2O flux observed from each agricultural plot in different seasons. Observations are segregated based on fertiliser application level on the agricultural plot. Fertiliser application level 0 refers to plots from the regular amount of fertiliser application, 300 N, and 1 refers to plots with reduced fertiliser application of 150 N.

4.2.2 The models

Using the mixed-models approach, six models were fit, with the same fixed effects but different random effects, to analyse various hypotheses regarding the data collected from the field experiment. The identity effect of each functional group is encoded as a fixed effect in all mixed-models. The models implemented as part of this work can be categorised into two sets. The first four models focus on analysing the strengths of various seasonal effects in the mixture, like the identity effects, diversity effects between the functional groups and their combinations. The fertiliser application levels are not considered in the first four models. The second set of models consider the fertiliser application levels and all communities of mixtures involved in the experiment. Fertiliser level is used as a random effect in these models to analyse the effect of the reduction in the fertiliser level on the N_2O emission. Refer to the methodology section 3.2.2 for the definitions of mathematical notations used to describe the various effects in the implementation details below.

Functional group effects

Identity effects and diversity effects between species from three different functional groups: grass (`G`), legume (`L`), and herb (`H`), in each seasonal observation of N_2O flux (flux) are analysed in the models presented below. First, the null model M0 is defined with the only intercept as the random effect. Then the next models (M1, M2, and M3) consider seasonal identity effects and diversity effects as random effects instead of the intercept. The models M0-M3 use the response collected from the plots which were applied with a reduced amount of fertilisers, communities 1-19 with 150 N fertiliser application per year, for fitting.

M0: Null model Null model corresponds to a null hypothesis in this experiment. This model was built based on the assumption that the N_2O emission is affected by the fixed identity effect (ID_{fix}) only. And identity effect and the diversity interaction effects are assumed to not cause any significant variations in the seasonal emission of the N_2O. This model is used as a base model used for comparison with other models. M0 was implemented in R using `lme4` package, as follows,

```
m0<-lmer(flux~G+L+H+(1|season), data, REML=FALSE)
```

Listing 4.1: M0: Null model

Where `flux` will be modelled as a function of the proportion of individual functional groups across all seasons, fixed identity effect (ID_{fix}), and an intercept for each season. Mathematical representation of M0 is shown in equation 4.1,

$$Y_t = \text{ID}_{\text{fix}} + \alpha_t + \eta, \text{ for } t = 1, ..., 4 \quad (4.1)$$

where, ID_{fix} represent a fixed identity effect as explained in equation 3.5 and α_t represent the the average deviation estimated around the fixed identity effect, ID_{fix}, for each seasonal measure t. t varies from $1, ..., 4$ corresponding to each of the seasons: spring, summer, winter, and autumn. The parameters estimated in this model are the coefficients in the D_{fix} and α_t.

M1: Seasonal identity effect model In this model, random identity effects are considered at seasonal levels. This model assumes that the identity effects of the functional groups are the significant causes for the variations in seasonal measurements of N_2O flux. Observe that the intercept is not included in the random effects of this model. Percentage of the functional groups are considered in this experiment, therefore, all coefficients in ID_{ran} for each season should sum up to the average seasonal change modelled in M0($alpha_t$). Here is the implementation of the model in R, where the identity effects in the mixtures are considered as both fixed and random effects.

```
m1<-lmer(flux~G+L+H+((0+G+L+H)|season),
           data, REML=FALSE)
```

Listing 4.2: M1: Seasonal identity effect model

In addition to M0, M1 will estimate the coefficients in the ID_{ran} instead of $alpha_t$. The mathematical form of M1 can be shown as follows,

$$Y_t = \text{ID}_{\text{fix}} + \text{ID}_{\text{ran}} + \eta, \text{ for } t = 1, ..., 4 \tag{4.2}$$

M2: Seasonal pairwise interaction model Seasonal diversity interaction effects (DE_{ran}) will be estimated in this model. The pairwise interaction effects encoded in DE_{ran} are assumed to be the major reason for seasonal variations in the response variable. M2 is implemented in R as follows

```
m2<-lmer(flux~G+L+H+((0+G:L+G:H+L:H)|season),
           data, REML=FALSE)
```

Listing 4.3: M2: Seasonal pairwise interaction model

where, terms `G:L`, `G:H`, and `L:H` represent the interaction terms, equivalent to GxL, GxH, and LxH. M2 will be estimating the coefficients of pairwise interaction terms in DE_{ran} along with the estimations of coefficients in ID_{fix}. Mathematical interpretation of M2 is shown in the equation below,

$$Y_t = \text{ID}_{\text{fix}} + \text{DE}_{\text{ran}} + \eta, \text{ for } t = 1, ..., 4 \tag{4.3}$$

M3: Seasonal full effects model Final model, M3, is a combined version of M2 and M3. It captures both the random identity effects (ID_{ran}) and random interaction effects (DE_{ran}) in each seasonal observation set of N_2O emission. M3 is implemented in R as follows,

```
m3<-lmer(flux~G+L+H+((0+G*L+G*H+L*H)|season),
           data, REML=FALSE)
```

Listing 4.4: M3: Seasonal full effects model

where, terms `G*L`, `G*H`, and `L*H` represent the pairwise interaction terms along with the identity terms. For example, `(G*L+G*H+L*H)` will be expanded as `(G+L+H+G:L+G:H+L:H)`. The model M3 will be estimating the coefficient in ID_{ran} and DE_{ran}, along with the coefficients in ID_{ran}, which is common in all models. Mathematically, M3 can be written as follows

$$Y_t = ID_{fix} + ID_{ran} + DE_{ran} + \eta, \text{ for } t = 1, ..., 4 \quad (4.4)$$

Functional group effects with fertiliser application levels

The amount of fertilisation application (fertiliser level) plays an important role in the variations of N_2O emission. Hence, the models presented in this section are designed to estimate the strength of the reduced fertiliser application levels(`FL`) on the N_2O emission. Results from the previous set of models M0-M1, discussed in chapter 5, shows that significant seasonal identity effects were observed in the experiment. Therefore, the model was implemented with seasonal identity effects and the seasonal effect of fertiliser application. Models M4 and M5 use the observations from all 20 communities for the analysis.

M4: Seasonal fertiliser level model The effect of reduced fertiliser application (FL_{ran}) on the seasonal emission of N_2O is modelled in M4. The fixed effects are same as the previous models, ID_{fix}. The random effects include an intercept and the coefficient for reduced fertiliser effect encoded as 1 for communities with reduced fertiliser application and 0 for communities with regular fertiliser application. The Intercept is included in the random effects to capture the possibility of other factors which would influence the N_2O emission. M4 assumes that the seasonal variations in N_2O emission is affected by the level of fertiliser application and an average effect of other unknown factors. Mixed-model implementation of M4 in R is shown below,

```
m4<-lmer(flux~G+L+H+(1+FL|season),
         data, REML=FALSE)
```

Listing 4.5: M4: Seasonal fertiliser level model

The mathematical representation of the model M4 is shown in the equation 4.5, which clearly shows the random effects and fixed effects considered in the model.

$$Y_t = ID_{fix} + \alpha_t + FL_{ran} + \eta, \text{ for } t = 1, ..., 4 \quad (4.5)$$

M5: Seasonal fertiliser level and identity effects model In the previous model, M4, the intercept was included as a random effect. The average effect of unknown factors is split into identity effects of the functional groups. Hence, the random identity effect (ID_{ran}) is considered instead of the intercept. In this model coefficient in FL_{ran}

estimates the effect of reduced fertiliser application and the coefficients in ID_{ran} estimate the strength of individual functional group identity effect for each seasonal observation set. The assumption of this M5 is that the seasonal variations in N_2O emission are affected by the fertiliser application level as well as the identity effects of individual functional groups. R implementation of M5 is shown below,

```
m5<-lmer(flux~G+L+H+((0+FL+G+L+H)|season),
          data, REML=FALSE)
```

Listing 4.6: M5: Seasonal fertiliser level and identity effects model

Mathematical formulation of M5 with fixed identity effects (ID_{fix}), random fertiliser level effects (FL_{ran}), and random identity effects (ID_{ran}) is shown in 4.6

$$Y_t = \text{ID}_{\text{fix}} + \text{FL}_{\text{ran}} + \text{ID}_{\text{ran}} + \eta, \text{ for } t = 1, ..., 4 \tag{4.6}$$

M6: Seasonal fertiliser level and full effects model In this model, random diversity interaction effects are included along with the random effects considered in M5. This model estimates coefficients in FL_{ran}, when represents the effect of reduced fertiliser application on the response variable. The coefficients of ID_{ran} and DE_{ran} estimate the identity effect and diversity interaction effect on the N_2O emission. The assumption of M6 is that seasonal variations in the N_2O emission are affected by fertiliser application, identity effects, and diversity interaction effects between multiple functional groups. R implementation of the model is shown as follows,

```
m6<-lmer(flux~G+L+H+((0+FL+G*L+G*H+L*H)|season),
          data, REML=FALSE)
```

Listing 4.7: M6: Seasonal fertiliser level and full effects model

Mathematical formulation of M6 with fixed identity effects (ID_{fix}), random fertiliser level effects (FL_{ran}), random identity effects (ID_{ran}), and random interaction effects (DE_{ran}) is shown in 4.7

$$Y_t = \text{ID}_{\text{fix}} + \text{FL}_{\text{ran}} + \text{ID}_{\text{ran}} + \text{DE}_{\text{ran}} + \eta, \text{ for } t = 1, ..., 4 \tag{4.7}$$

4.3 Diversity-Interactions model implementations

In the data collected from the experiment, spikes are observed in the emission of N_2O, in multiple types of mixture communities around the time of application of the fertiliser. The spikes indicate the possibility of significant changes in the dynamics of mixture interactions during the time of the fertiliser application. DI modelling framework was used to fit the data aggregated based on the time of fertiliser application.

4.3.1 Aggregation based on the time of fertiliser application

To study the changes in the dynamics of various effects in the mixture of species, N_2O flux observed immediately after the fertiliser application can be aggregated and analysed as repeated measure data. The fertiliser was applied five times throughout the experiment with varying intervals as indicated in (Appendix B). Therefore, five sets of observations of N_2O flux need to be aggregated, which results in five repeated measures of aggregated N_2O flux for each plot. As explained in the previous section 3.1, N_2O flux was collected at a higher frequency of four times a week for two weeks immediately after the fertiliser application. Hence, total N_2O flux observed over 2 weeks after the fertiliser application was used for the repeated measure analysis. DI modelling method was used to model the interactions in the mixture. After the aggregation of eight observations immediately after a fertiliser treatment, the data which will be used for fitting DI models can be visualised as shown in the image 4.2. Note that the fertiliser application time (T1, T2,..., T5) is different from the fertiliser application level, which refers to the amount of fertiliser applied.

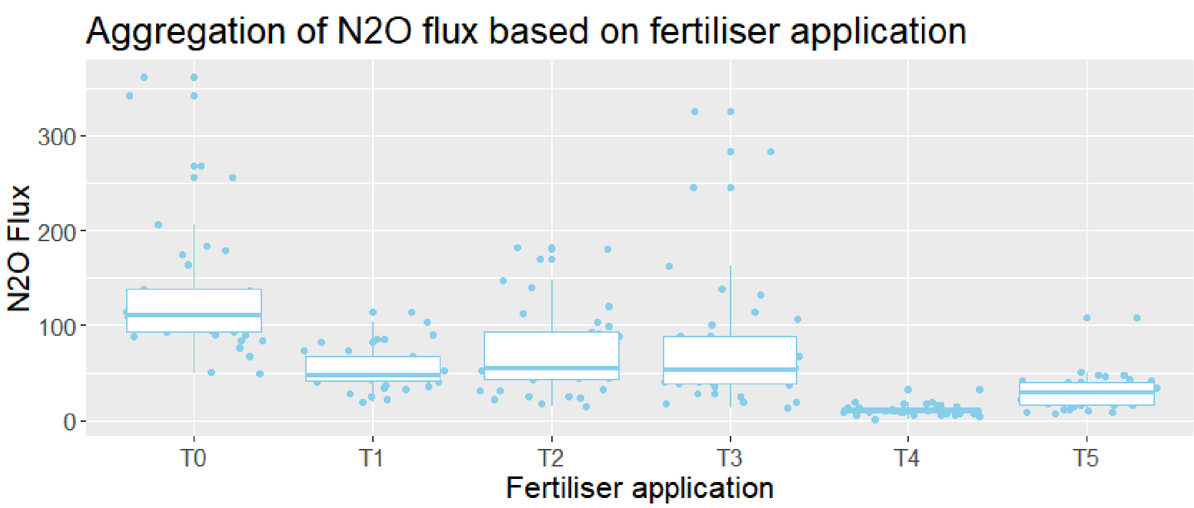

Figure 4.2: Total N_2O flux observed in eight observations after the the fertiliser application. T1, T2, T3, T4 and T5 indicates the five applications of fertiliser. Only plots with reduced fertiliser application of 150 N is aggregated.

The visualisation of the data image 4.2, aggregated based on fertiliser application has noticeable outliers. Hence, RM-ANOVA can not be applied for this data, as the response variable, aggregated N_2O flux, is not normality distributed. To analyse the changes in the various effects involved in the mixtures, multiple DI models were fit for each aggregation set T1, T2,..., T5 and their coefficients were compared along with their significance using the p-value.

4.3.2 The models

DI modelling framework, available in the R package `DIModels`, was used to fit the aggregated observations collected immediately after each fertiliser application. Total five models were fit for five times fertiliser application. 'Full effects' model in DI modelling captures both identity effects and pairwise interaction effects. Therefore, the 'Full effects' approach is used in the following implementations. Observations from communities 1-19 with reduced fertiliser levels were used for fitting as this analysis focuses on analysing the changes in the dynamics of mixture effects with repeated applications of fertiliser at the same level.

Implementation of DI model in R is shown below,

```
full_di <- DI(y = "flux",
              prop = c("G","L","H"),
              FG = c("G","L","H"),
              data = data,
              DImodel="FULL")
```

Listing 4.8: DIM: Diversity-interactions model implementation

Mathematical representation of the model is shown in the equation 4.8,

$$Y = \mathrm{ID} + \mathrm{DE} + \alpha \tag{4.8}$$

Where ID represents the identity effects and DE represents the diversity interaction effects between functional groups in the mixture. and $\alpha \sim \mathcal{N}(0, \sigma^2)$ represents the error term. The coefficients involved in identity effects and diversity interaction effects are explained in 3.2.1.

Chapter 5

Evaluation

The models were designed and fitted based on various assumptions using a variety of parameters. This section covers the evaluation of the data and the results from the models fitted in the previous section. First, the explanatory analysis of the data is reported. It covers a brief overview of the original data collected from the experiment and visualises the aggregated data after the preprocessing steps explained in 4.1. Next, the seasonal analysis of the diversity effects reports the results from mixed-models, which estimates the seasonal variations in the diversity effects on the N_2O emission. Later, the evaluations of DI models were analysed to study the changes in diversity effects based on repeated fertiliser applications. Finally, a summary of all different kinds of evaluations explained in the section are presented.

5.1 Explanatory analysis of the data

A brief overview of the original data collected from the field experiment, is shown in the figure 5.1. To provide a cleaner overview of data, average N_2O flux observed over the time of the experiment from different groups of communities were are plotted. Monocultures are communities which contain species of only one kind of functional group. For example, grass monocultures consist of either one or both grass species, L. perenne and P. pratense. Mixed cultures contain species from at least two different functional groups. 300 N grass monocultures are the set of control communities, which simulate the regular agricultural practice. Fertiliser application date is marked with a black arrow at the top of the plot. The figure reported in Cummins et al. (2021) plots the same data based on the groups created with species monocultures and mixtures. Whereas, the figure 5.1 plots the data based on the groups created with functional group monocultures and mixtures.

A quick glance at the plot 5.1 shows that a higher amount of N_2O emissions were

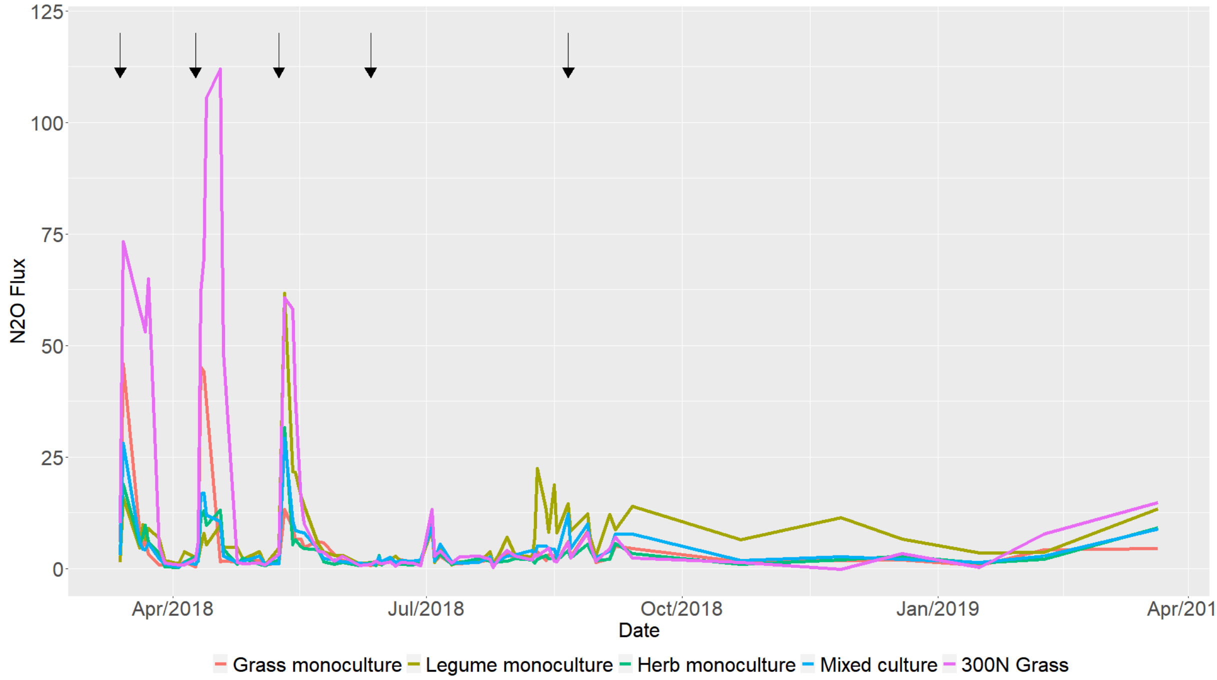

Figure 5.1: Average N_2O Flux from different groups of plots. Plots are grouped as *Grass monoculture* - containing only grass species, *Legume monoculture* - containing only legume species, *Herb monoculture* - containing only herb species, *Mixed culture* - containing at least two different species, *300N Grass monoculture* - Grass monoculture plots which were applied with higher mount of fertiliser. Black arrows at the top of the figure mark the time of fertiliser application date

observed from community 20, to which a higher amount of fertiliser (300 N) was applied. Hence, there is a good possibility of significant change in N_2O emissions based on the amount of fertiliser applied to the monocultures. Spikes in the N_2O emissions are observed in almost all groups around the time of fertiliser application, and the height of the spike changes differently for different monocultures and mixtures, with repeated fertiliser application. This behaviour of the spikes N_2O emissions suggests that the dynamics of the diversity effects in the plots changes with multiple applications of the fertiliser. However, fertiliser application may not be the only factor causing the changes in spike, it could be based on the seasonal changes in the environment or other factors like soil minerals, microbial activities as well. Data was collected more frequently at around the first six months of the experiment then the last six months. However, low N_2O emissions were expected as the fertiliser was not applied in the autumn and winter seasons and the possibility of emission is also less because of the low temperatures.

The original data was preprocessed in two different ways, based on the modelling framework and the factors considered for the analysis. Data preprocessing is explained in the section 4.1. One of the preprocessing steps aggregated the observations based on seasons: spring, summer, autumn, and winter. Seasonally aggregated data was used to fit mixed-models to analyse a variety of effects, like seasonal effects, overall diversity effects, and the fertiliser application levels. In another way of preprocessing, a set of observations collected immediately after the fertiliser application were aggregated. Repeated measure data created as a result of aggregation based on fertiliser applications, were used to fit DI models to analyse the possible changes in the dynamics of the diversity effects in the spikes generated around the time of fertiliser application.

5.2 Seasonal analysis of the effects

Mixed-models were used to analyse the seasonal effects that could be responsible for the variations in the emission of N_2O. Model implementation details, like hypothesis and the mathematical representation, are discussed in section 4.2. As explained before, two kinds of seasonal analysis were implemented. First analysis was focused on the various combinations of diversity effects as random effects. And the second analysis was focused on analysing the effect of reduced fertiliser application of 150 N, compared to the regular fertiliser application on a grass monoculture. Fixed effects were the same in all models to simplify the comparison of multiple mixed-models.

5.2.1 Diversity effects

Models M1-M3 were fitted to the observations from plots 1-19, which received a reduced amount of fertiliser. Each model was evaluated using the evaluation method discussed in methodology chapter 3.3 to compare the models against the baseline null model, M0. For comparison, the null model was also fitted using the same data, which was used for fitting M1-M4. Goodness-of-fit measures of the models are tabulated in table 5.1 below.

Model	Random Effects	AIC	BIC	Deviance	p-R2
M0	α	-429.6	-414.5	-439.6	0.486
M1	`G+L+H`	-456.1	-425.8	-476.1	0.625
M2	`G:L+G:H+L:H`	-355.17	-324.94	-375.17	0.183
M3	`G+L+H+ G:L+G:H+L:H`	-435.71	-360.11	-485.71	0.681

Table 5.1: Comparison of the mixed-models to analyse seasonal diversity effects

The model M3, which considers functional group identity effects and interaction effects as random effects, is the best fit for the data as pseudo R^2 value 0.681, is the highest compared to other models reported in 5.1, which indicates that the model accounts for the parameters which causes 68.1% variations in the response variable, N_2O flux. Moreover, deviance (-485.71) of the model M3 is also the lowest. However, pseudo R^2 value (0.625) and the deviance (-476.1) of the model M1 are very close to the values of the model M3, with better (lower) values of AIC (-456.1) and BIC(-425.8) compared to model M3. Penalties are applied for the models with higher number of parameters in AIC and BIC calculations. AIC (-435.71) and BIC (-360.11) values of model M3 are very close to the values of model M1. Hence, considering the higher pseudo R^2, model M3 can be considered to be the best estimate the seasonal diversity effects on the N_2O emission.

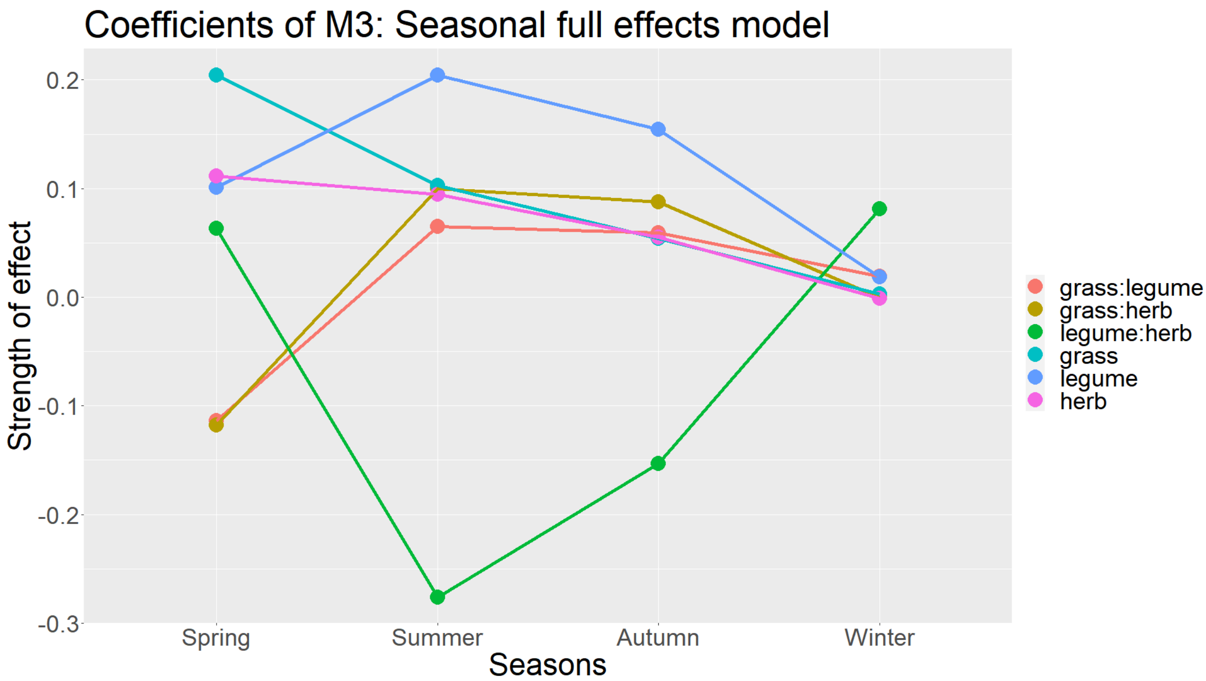

Figure 5.2: Coefficients of M3: Seasonal full effects model

The coefficients estimated for model M3, which considers both fixed and random effects, are plotted in the image 5.2. In the plot, y-axis is the coefficient estimate, which indicates the strength of the parameter on the estimation of the response variable. The dynamics of changes in the strength of each parameter for different seasons can also be observed in the plot. The diversity interaction between legumes and herbs is estimated to affect negatively to N_2O emission in summer and autumn. Mixture containing grass has an estimated negative effect of interactions on the emission in the spring, however they contribute positively in other seasons. Identity effect of grass species is estimated to reduce throughout the year. Note that these estimations are from the communities with reduced fertiliser application only. Adding the fertiliser application levels in the mixed model would possibly improve the estimations from the model.

5.2.2 Fertiliser application effects

The models M4-M6 were fitted using observations from all communities and different fertiliser application level was considered in these as a random effect. Baseline model M0, which accounts for only the fixed identity effect (ID_{fix}) was used for the comparison to evaluate the goodness-of-fit. The model M0 was refitted with observations from all communities to correctly compare the fitness parameters with the models M4-M6. Evaluation of the goodness-of-fit parameters as listed in table 5.2.

Model	**Random Effects**	**AIC**	**BIC**	**Deviance**	**p-R2**
M0	α	-259.63	-244.01	-269.63	0.318
M4	`1+FL`	-415.06	-393.19	-429.06	0.779
M5	`G+L+H+FL`	-436.54	-392.81	-464.54	0.845
M6	`G+L+H+ G:L+G:H+L:H+FL`	-407.51	-307.54	-471.51	0.862

Table 5.2: Comparison of the mixed-models to analyse seasonal diversity effects along with fertiliser application level.

Adding fertiliser level as a random effect in the model improves the pseudo R^2 from 0.318 (in baseline model M0) to 0.779 in model M4. Hence, the fertiliser application level itself accounts for the 77.9% of variation in the emissions of N_2O. Best pseudo R^2 value of .862 is observed in model M6 along with the lowest deviance of -471.51. The model M5 has pseudo R^2 value of 0.845 and the deviance of -464.54, which are very close to the same parameter values of M6. AIC and BIC values of model M6 (-407.51, -307.54) are greater than model M5 (-436.54, -392.81), which can be explained based on the number of parameters used for fitting. Compared to model M5, random diversity interaction effects are included in model M6. Diversity interactions are one of the important effects in a mixture environment and are not expected to cause overfitting when the experiment was conducted in similar conditions as the same kind of interactions can be expected from any two species in a given environmental condition. Thus, model M6, which estimates the N_2O emission based on fixed identity effect, random identity effect, random interaction effects, and the effect of fertiliser application level, is considered to be the best model to explain the seasonal dynamics of N_2O emission.

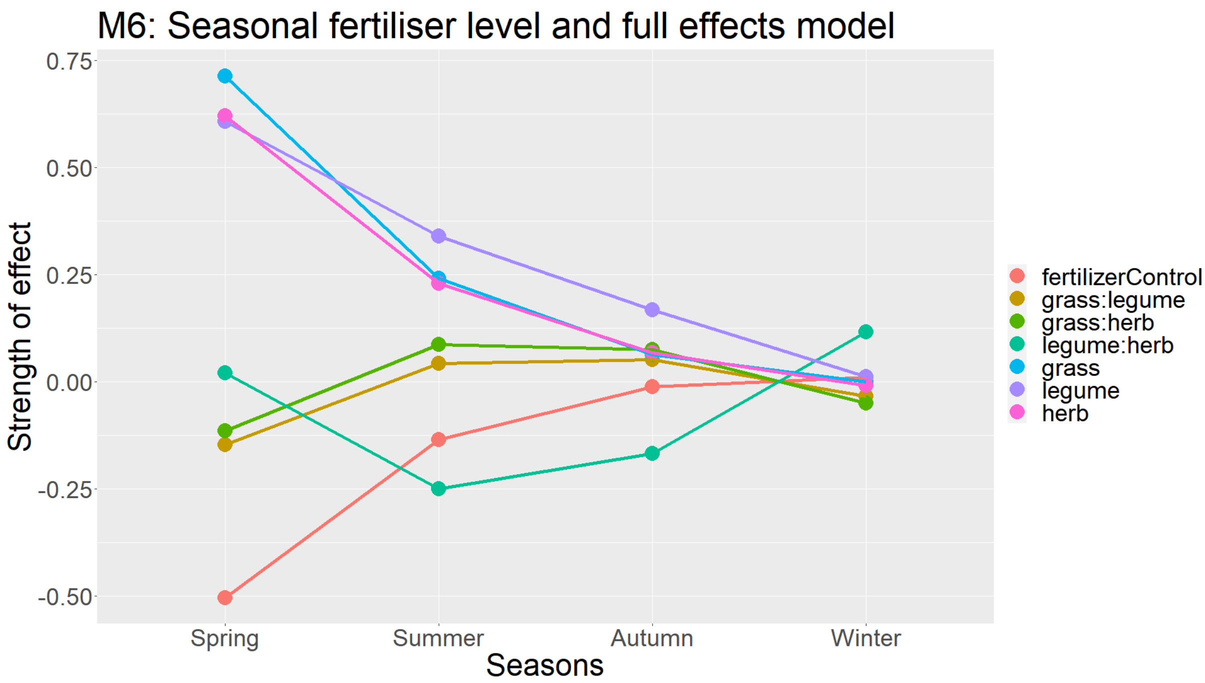

Figure 5.3: Coefficients of M6: Seasonal fertiliser level and full effects model

Overall coefficients (fixed effects + random effects) estimated in model M6 are plotted in 5.3. Strength of the effect of reduced fertiliser application is estimated to contribute highly to the reduction in N_2O emission. Hence, reducing the amount of fertiliser application is necessary to minimise the N_2O emissions. Identity effects were reduced along the time but they contributed positively to the response variable. Strength of the pairwise interaction varies in small interval throughout the season, hence the interaction effects are estimated to contribute weakly to the reduction of N_2O emissions.

5.3 Analysis of the diversity effects based on the fertiliser application

DI models were fitted to the aggregated observations collected immediately after the fertiliser application. Therefore, five full effects models, which considered the identity effect and interaction effects, were fit to the observations collected from communities 1-19, the experiment plots which were applied with reduced fertiliser levels. Five models are named as DIM1, DIM2, DIM3, DIM4, and DIM5, where DIMn, the model fitted to the aggregated observations collected after n^{th} fertiliser application. The estimated coefficients from the DI models, which were fit after each fertiliser application are shown table 5.3. No significant interactions between species from different functional groups were observed, except one significant interaction observed between grasses and herbs after the third application of the fertiliser. The significant interaction between grasses and herbs has a very high impact (-403.688) on the reduction of N_2O emissions. However, this effect is not observed consistently, so the overall significant interaction effect between any functional groups can not be established.

Model	grass	legume	herb	grass:legume	grass:herb	legume:herb
DIM1	**75.139***	**47.355***	**49.937***	-74.107	-6.079	12.625
DIM2	**133.051***	**35.899***	**56.668***	-64.796	-117.826	57.235
DIM3	**48.220***	**146.102***	**60.750***	143.202	91.132	**-403.688***
DIM4	**10.461***	**11.018***	**10.273***	-0.925	6.974	6.910
DIM5	**24.299***	**44.599***	**17.769***	-10.450	44.648	18.007

Table 5.3: Coefficients of the DI models fit to the aggregated observations collected after fertiliser application. Numbers presented in bold are the estimates of the significant effect ($p < 0.05$)

5.4 Summary

By explanatory analysis of the data, seasonal changes in the diversity effects and fertiliser application level (reduced: 150 N and regular: 300 N) were assumed to be the major factors influencing the N_2O emissions. The spikes in the N_2O flux after the fertiliser application was also an point of interest, as the variations in the response from the different kind of communities in the experiment was not same in all spikes. The mixed-model with random effects that include identity effects, diversity effects, and fertiliser level effects, were found to account for the 86.2% variations observed in the N_2O flux. The coefficient plot of the best mixed-model M6, shows that seasonal strengths of identity effect are closely correlated. It also explains that, reduced fertiliser application amount is strongest factor responsible the reduction in N_2O emissions, and second strongest factor is the interactions between legume and herbs, but only during summer and winter. Fitting DI models on observations immediately after fertiliser application did not show any significant diversity interactions among the functional groups in the mixture. Except for the interactions between legume and herb after third fertiliser application, which is not consistent for all fertiliser applications.

Chapter 6

Future Work

Reduced amount fertiliser application was identified as one of the strongest effects that influenced N_2O emissions in this experiment. Reduction in fertiliser will affect the yield of the plot. Hence, yield from the agricultural plot is considered for the analysis to identify the ideal amount of fertiliser reduction required without compromising on the yield. Time based analysis of N_2O emission can be extended to analyse various other factors like, soil minerals, microbial activities, livestock grazing, type of fertiliser usage, that would have a major effect on the N_2O emission. Research works already considered some of the factors discussed here. However, variation in those factors along the time is not widely considered. Hence, analysing the important factors that affect the N_2O emission, with the consideration of changes in those factors along the time, like seasonal or based on an external treatment, would be helpful in understanding the dynamics of N. In turn, it enables considering the changes required in the current agricultural practice specific to a particular time or season to achieve further reductions in N_2O emissions.

Multi species mixture analysed in this experiment was observed in a very controlled environment. Hence, the results achieved from this analysis may not be a very robust prediction. Actual emissions would vary in practical cases. Therefore, conducting multi-species mixture experiments with the consideration of a wide variety of important factors like different soil types, different geographical locations, and effects of livestock grazing, etc would be necessary for a robust analysis.

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Appendix

Appendix A

Community	Reps	FGs	Species	FG(Grass)	FG(legume)	FG(deep)	L.p	P.p	T.p	T.r	C.i	P.l
1	3	1	1	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
2	3	1	1	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
3	3	1	1	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
4	3	1	1	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
5	3	1	1	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00
6	3	1	1	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00
7	2	1	2	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00
8	2	1	2	0.00	1.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00
9	2	1	2	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.50	0.50
10	2	2	4	0.50	0.50	0.00	0.25	0.25	0.25	0.25	0.00	0.00
11	2	2	4	0.50	0.00	0.50	0.25	0.25	0.00	0.00	0.25	0.25
12	2	2	4	0.00	0.50	0.50	0.00	0.00	0.25	0.25	0.25	0.25
13	1	3	5	0.60	0.20	0.20	0.60	0.00	0.10	0.10	0.10	0.10
15	1	3	5	0.20	0.60	0.20	0.10	0.10	0.60	0.00	0.10	0.10
16	1	3	5	0.20	0.60	0.20	0.10	0.10	0.00	0.60	0.10	0.10
17	1	3	5	0.20	0.20	0.60	0.10	0.10	0.10	0.10	0.60	0.00
18	1	3	5	0.20	0.20	0.60	0.10	0.10	0.10	0.10	0.00	0.60
19	3	3	6	0.33	0.33	0.33	0.17	0.17	0.17	0.17	0.17	0.17
20	4	1	1	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00

Table 1: Mixture design of the experimental sites with various combination of species proportions.

Appendix B

Fertiliser was applied 5 times in the experiment to the communities listed in 6. Plots belonging to communities 1-19 were applied with reduced fertiliser amounts, and plots of community 20 were applied with regular fertiliser amounts, which reflect the current agricultural practice.

Split	Date	Fertiliser Application	
		Experiment sites (Community 1-19)	**Control sites** (Community 20)
1	12-Mar-2018	30 kg N ha^{-1}	60 kg N ha^{-1}
2	09-Apr-2018	30 kg N ha^{-1}	60 kg N ha^{-1}
3	09-May-2018	30 kg N ha^{-1}	60 kg N ha^{-1}
4	11-Jun-2018	20 kg N ha^{-1}	40 kg N ha^{-1}
5	20-Aug-2018	40 kg N ha^{-1}	80 kg N ha^{-1}

Table 2: Details of fertiliser application to the experimental site.

Appendix C: Statistical significance

Statistical significance was formally introduced by Fisher (1992), and it is one of the most accepted evaluation parameters for hypothesis testing. The effects considered in the hypothesis are considered as significant if the *p-value* is less than a very small value α, called significance level. The hypothesis will be accepted over a null hypothesis if a model that is fitted with assumptions based on a hypothesis has significant effects ($p \leq \alpha$), otherwise it will be rejected. An illustration of p-value is shown in the following image 1.

Test-statistic like z-test, t-test, F-test, and chi-squared test and many more tests can be used to calculate *p-value* based on the distribution of the variable (Wikipedia (2021)).For a test statistic t from a distribution T, two-sided tail test can be calculated as shown in equation 1 below,

$$p = 2 * min\{Pr(T \geq t|H_0), Pr(T \leq |H_0)\} \tag{1}$$

Where H_0 indicates the given null hypothesis.

Significance level α should be chosen before the data collection depending on the context of the study. However, a standard *p-value* of 0.05 is widely used. Fixed value of 0.05 is debated among the researchers as some of the experiments are hard to reproduce and the misinterpretation of *p-value* with $p \leq \alpha$ would lead to biased results with no practical significance.

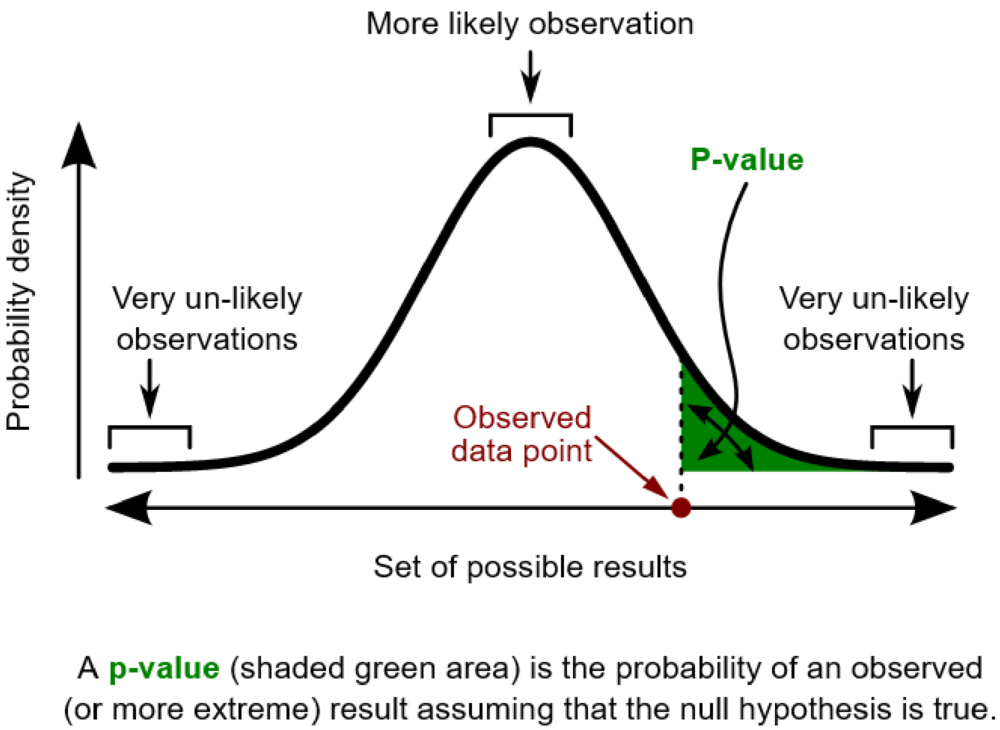

Figure 1: Visual illustration describing the meaning of the *p-value* as explained in Wikipedia (2021)