Effect of Pasture Management System Change on In-Season Inorganic Nitrogen Pools and Heterotrophic Microbial Communities

Maciej Musiał 1, Jan Kryszak 1, Witold Grzebisz 2,*, Agnieszka Wolna-Maruwka 3, and Remigiusz Łukowiak 2

1 Department of Grassland and Natural Sciences, Division of Ecosystems Biodiversity, Poznan University of Life Sciences, Dojazd 11, 60-632 Poznan, Poland; maciejmusial@gmail.com (M.M.); jan.kryszak@up.poznan.pl (J.K.)
2 Department of Agricultural Chemistry and Environmental Biogeochemistry, Poznan University of Life Sciences, Wojska Polskiego 71F, 60-625 Poznan, Poland; witold.grzebisz@up.poznan.pl (W.G.); lukowiak@up.poznan.pl (R.Ł.)
3 Department of General and Environmental Microbiology, Poznan University of Life Sciences, Wojska Polskiego 28, 60-637 Poznan, Poland
* Correspondence: amaruwka@up.poznan.pl

Received: 16 April 2020; Accepted: 14 May 2020; Published: 18 May 2020

Abstract: It has been assumed that the system of long-term pasture management exerts a significant impact on the soil microorganisms count, subsequently affecting the availability of mineral nitrogen ($N_{\text{min}}$). This hypothesis was tested in an experiment on a long-term pasture with two distinct systems of grass sward management, i.e., grazing and mowing. Mowing significantly increased the microorganisms count by 13%, 28%, 86%, and 2% for eubacteria (EU), actinobacteria (AC), molds (MO), and Azotobacter (AZ), respectively. The content of $N_{\text{min}}$ decreased throughout the vegetative growing season, reaching its lowest value after the 3rd grazing cycle. The impact of microorganisms on the $N_{\text{min}}$ pools increased in the order: molds < eubacteria < actinobacteria. The count of actinobacteria in the alkaline organic soil increased in response to drought, contribution of Dactylis glomerata L. in the sward, and the shortage of available phosphorus. The sound pasture management system is possible by introducing alternate grazing and mowing cycles. The core of sustainability is the enhanced activity of actinobacteria after changing the system from grazed into mowed.

Keywords: grass sward; management; soil microbial community; available nutrients; mineral nitrogen

1. Introduction

Grasslands are a natural source of high quality forage, rich in energy, proteins, and nutrients [1]. Pasture positively affects the condition of grazing animals and significantly reduces the costs of milk production. Study of the relationship between grazing animals and sward quality and its productivity is one of the fundamental research areas in countries with a high level of animal husbandry [2,3]. The productivity of natural or extensively fertilized grasslands depends on the availability of plant nutrients within the consecutive mowing or grazing cycle. Under these conditions, nutrient availability, especially nitrogen (N) to the sward plants, depends to a great extent on the intensity of organic matter decomposition. Hence, composition and activity of soil microbial communities seem to be a decisive factor for controlling, or even enhancing grassland productivity [4].
In Poland, on many farms focused on milk production, interest in an efficient pastoral economy is high [5]. Grassland in Poland occupies about 20% of the agricultural area (3.271 mln ha, including 0.774 mln pastures). The productivity of natural grasslands is low, largely depending on the natural soil productivity and distribution of precipitation [6]. The used grasslands are managed in two different ways. The principal management system is mowing, the grass then being used for the production of hay or silage for cows. A less common system is grazing, mainly of milk cows. Two grazing systems dominate in pasture management in Poland. The continuous grazing on a pasture is typical for small farms. The rotational grazing is applied by the medium intensive milk producers [7].

In agricultural production, the processes of mineralization of organic matter play an important role, contributing to the natural source of mineral elements to sward plants. The soil microbiome consists of heterotrophic organisms, including bacteria, actinobacteria, and microscopic fungi [8]. Heterotrophic microorganisms play a major role in the mineralization of organic matter, derived from plant residues or animal excrement. The effectiveness of the transformation of these compounds by soil microorganisms to build their own cells depends not only on the carbon quality and quantity, but also on the condition of environmental factors, such as soil humidity, ambient temperature, precipitation, and the availability of N [9]. The most important groups of microorganisms decomposing an organic substance include eubacteria. For farmers, the importance of bacteria results from their effect on the intensity of mineralization processes of organic matter, both native and that which is introduced into soil as plant residues or natural fertilizers. Among the soil bacteria, the genus *Azotobacter* sp. plays a special role which is widely recognized as an indicator of soil fertility. This genus inhabits fertile soils rich in mineral and organic colloids, providing a soil reaction close to neutral [10]. The number of *Azotobacter* sp. cells depends on the soil type and its pH value, which may range from 0–1000 cfu g$^{-1}$ (colony forming units). In acidic soils with a pH below 6, these microorganisms are absent or present in a very small number [11].

Actinobacteria is the group of microorganisms, which has a naturally high potential to transform organic compounds that are difficult to decompose, such as chitin, lignin, higher fatty acids, steroids, and humic acids [12,13]. In fertile soils, the number of these microorganisms is smaller than eubacteria and their quantitative ratio is approximately 40:60. In dry soils, the number of actinobacteria increases and may exceed the population of bacteria [14]. Fungi also play an important role in the flow of energy and the circulation of nutrients in the soil. There is a lot of scientific evidence that molds are powerful factors in the processes that change the geochemical properties of soil. The wide surface–volume ratio of fungi affects the intensity of compound exchange with the environment [15–17]. A dominance of fungi over bacteria indicates a deterioration in the conditions of the soil environment [18]. Soil microorganisms through their influence on the transformation intensity of the soil organic matter by the processes of mineralization and immobilization of nutrients, significantly shape their circulation in the soil/plant system [19,20]. Zhao et al. [21] documented that microbial composition and activity in low-fertile soils significantly depended on grazing intensity, including both animal excrement and plant residues. The effect of grazers on the composition of the soil microbiome should also include other factors, such as trampling, which significantly affects soil physical properties [22].

The intensity of pasture sward mowing or grazing affects the quantity and quality of organic matter input, and thus the rate of its transformation in the soil. In the grazing system, the urine and feces left by animals directly on the sward lead to an increase in the content of labile forms of organic matter and nitrogen in the soil [4]. Both of these groups of factors directly and indirectly affect the microbial activity of the grassland soils. The release of $N_{\text{min}}$ depends on the potential of soil microorganisms to mineralize its organic compounds to ammonium [23,24]. So far, the effect of meadow management on the release of inorganic N and other plant nutrients poorly recognized [25]. Although there have been a number of studies on the effects of mowing and/or grazing on the microbial community structure [18,21,26], but only a few have focused on the relationships between microbial diversity in the pasture soil and the contents of the available nutrients [27,28].
Pastures are the main source of feed for ruminants over the world. The intensification of dairy cow production, especially in leading countries in Europe, decreases, but does not eliminate, the need for feed supply from pastures [29]. The natural methods of pasture sward use are periodical grazing/resting cycles. During the grazing period, animals expel excreta, which are rich in all plant nutrients, but especially in N, directly onto the pasture sward. The expected production effect of N in excreta is its direct impact on growing plants and indirect influence on soil N pools. A high N concentration, especially in urine patches, results in ammonia volatilization (N-NH\(_3\)) directly into the atmosphere [30]. The second source of easily mineralized N, left on a sward after a grazing period, are sward litters. The transformation pathways of these N pools in the sward soil and their subsequent impact on soil N pools are very complex [31]. Easily mineralizable N significantly impacts the activity of soil microorganisms. As a consequence, the stable organic carbon pool is enriched in organic compounds of microbial origin [32]. The narrowed C:N ratio, especially in organic soil, results in an enhanced increase of N mineral pools, i.e., ammonia (N-NH\(_4\)), and nitrate (N-NO\(_3\)). During the vegetative season, both pools are a direct source of N to the growing plants. However, during winter or early spring, nitrates undergo leaching, subsequently disturbing the functioning of water ecosystems [33,34].

The management concept known as sustainable intensification of agriculture seems to be an adequate approach to a sound use of pastures. The key advantage of the grazing system is the relatively low cost of milk or meat productivity. The main disadvantage of this system is the great threat of nitrate leaching to water ecosystems [35]. A short-term change in the grazing system by temporally implementing the mowing seems to be the simplest way to exploit easily available N pools, while maintaining the previous production level of feed for animals, concomitant with the simultaneous protection of the neighboring ecosystems.

The main objective of this study was to evaluate the impact of heterotrophic microbial communities, taking into account variable environmental conditions (weather) in two management systems of long-term pasture, i.e., grazing and mowing, on the in-season variability of inorganic nitrogen pools and the availability of other plant nutrients.

2. Materials and Methods

2.1. Site, Soil, and Climate

The research was conducted at Brody, an experimental station belonging to Poznań University of Life Sciences. The farm is located about 50 km west of Poznań. From the northwest, it borders the Bolesława Papi Reserve with lake Zagierzyneckie. The natural grassland covers 160 ha, of which 40 ha is used as a pasture that is divided into 12 quarters. The research was carried out in 2006, 2007, and 2008 on quarter No. 2. The sward of this quarter before the experiment was dominated by three groups of species, representing grasses (61%), legumes (9%), forbs (10%), and weeds (quack grass–9% and dicotyledonous species–11%). The key grass species, i.e., perennial ryegrass and common meadow-grass covered 60% of the sward area. The geographical location of the studied pasture was: entry to the accommodation unit—N 52° 25′ 59.42″ E 16° 17′ 19.30″, central point—N 52° 25′ 58.62″ E 16° 17′ 11.67″. The investigated pasture is classified as medium wet grassland (bonitation class IV) [36]. It was established on muck soil originated from low peat, dark gray (0–40 cm). The second horizon occupies low peat very strongly decomposed, almost black (40–65 cm). Beneath the organic layers, silty clay occurs. Prior to the initiation of the study, the soil contained 35% of organic matter in the 0–30 cm layer, and had moderate drainage.

The local climate, classified as intermediate between atlantic and continental regions, is seasonally variable, particularly during the summer [37]. Analysis of average air temperatures during the research showed a significant difference in annual mean temperatures as compared to the long-term average (1961–2005) of 13.4 °C during the vegetative growing season (months 4–10) (Figure 1). The mean temperature during each grazing cycle was 15.9, 14.6, and 15.1 °C for 2006, 2007, and 2008, respectively. The average temperature for the consecutive grazing cycle showed a typical annual variation (Table 1;
Figure 1). The warmest grazing cycle was summer 2006, when the average temperature reached almost 23 °C; concomitant with a shortage of precipitation which resulted in severe drought. These two courses of meteorological events led to a grass “burnout”, finally resulting in a significant change in the grass species composition (Table A1). The total sum of precipitation was highly variable, especially during the summer months (Table 1; Figure 1).

2.2. Experimental Design

The study was conducted on a rectangular pasture quarter of 100 × 300 m, 3.0 ha in size. The experiment was arranged as a two-factorial design. The first factor was the number of grazing/mowing (acronym Gr/Mo) cycles during each growth season (from May to October, i.e., 1, 2, 3, and 4 cycles, see Table 1 for detailed dates). The second factor was the sward management system, i.e., grazing or mowing. Grazing duration was determined, depending on the grass yield, based on the yearly stocking density (SD) of 5.94 ha⁻¹ Livestock Unit (LSU). Total SD was constant within each grazing event and between years, reaching on average 72 SD per 3 ha per day. The grazing

Table 1. The calendar date of soil sampling and basic meteorological data in the period preceding grazing in respective years.

| Year | Sampling Date and Meteorological Data |
|------|---------------------------------------|
|      |                                       |
| 2006 | 19.5 29.06 11.08 25.10                |
|      | Temperature. °C ± SD                  |
| 2006 | 10.5 ± 4.0 17.9 ± 4.8 22.7 ± 3.7 14.8 ± 3.5 |
|      | Precipitation. mm                     |
| 2006 | 83.1 33.6 206.3 131.7                 |
| 2007 | 8.05 11.7 14.08 19.10                 |
|      | Temperature. °C ± SD                  |
| 2007 | 8.8 ± 3.5 17.5 ± 3.5 19.3 ± 3.5 13.3 ± 3.7 |
|      | Precipitation. mm                     |
| 2007 | 107.9 332.5 117.0 73.4                |
| 2008 | 19.06 22.07 26.08 19.11               |
|      | Temperature. °C ± SD                  |
| 2008 | 13.3 ± 5.1 19.2 ± 2.2 20.1 ± 3.1 11.2 ± 4.1 |
|      | Precipitation. mm                     |
| 2008 | 142.6 74.5 154.7 125.2                |

SD—standard deviation of temperature within consecutive sampling date; the spring regrowth date = 5 °C.

2.2. Experimental Design

The study was conducted on a rectangular pasture quarter of 100 × 300 m, 3.0 ha in size. The experiment was arranged as a two-factorial design. The first factor was the number of grazing/mowing (acronym Gr/Mo) cycles during each growth season (from May to October, i.e., 1, 2, 3, and 4 cycles, see Table 1 for detailed dates). The second factor was the sward management system, i.e., grazing or mowing. Grazing duration was determined, depending on the grass yield, based on the yearly stocking density (SD) of 5.94 ha⁻¹ Livestock Unit (LSU). Total SD was constant within each grazing event and between years, reaching on average 72 SD per 3 ha per day. The grazing
period differed in the length, and lasted 1.0 to 5.0 days in 2006; 2.5–4.0 days in 2007, and 3.0–4.0 days in 2008. The daily length of the grazing event was four hours, lasting from 7.30 to 11.30 a.m. Sward was mowed by lawn mower Pasquali (100 cm single blade) at the end of each grazing event. Four replicated experimental plots of 30 m² were established for each management treatment along a transect. The distance between each plot from its centre was 35 m. This distribution was chosen due to the random movement of animals during grazing (Figure 2). The study on the content of Nₜₚₐₜ, soil available nutrients, and the soil microbiome composition was carried out on specially established subplots (four, 1 × 2 m in size) within each main plot.

The annual rates of fertilizer N as ammonium nitrate were 35 kg ha⁻¹, applied before the 1st and the 3rd grazing cycle (total 70 kg ha⁻¹). Phosphorus fertilizer was not applied. Potassium was applied at the beginning of the vegetative growing season in the form of 40% K₂O fertilizer and a dose of 40 kg K₂O ha⁻¹.

2.3. Soil Sampling and Analysis

Soil was sampled four times per year, one after each Gr/Mo event. (Table 1). Twenty 1.5-cm-diameter individual soil cores were collected per each subplot of the main plot at 0–15-cm depth and then composited.

Samples were sieved to <2 mm and dry stored prior to analysis. Subsamples (100 g) for mineral nitrogen determination were deep-frozen (−20 °C). For Nₜₚₐₜ determination, 20 g of each soil sample was shaken for 1 h with 100 mL of 0.01 M CaCl₂ solution (soil/solution ratio 5:1; m/v).

Concentrations of NH₄-N, NO₃-N were determined with the colorimetric method using flow injection analyses [38]. Soil samples for determination of available nutrients (P, K, Mg, Na) were air-dried and crushed to pass a 2-mm mesh size. The extractable nutrients were determined using 0.5 M HCl solution [39]. The content of available P in the extract was determined calorimetrically [40], while contents of K, Mg, and Na were determined using a flame type atomic absorption spectrometer [41].

2.4. Soil Microbiome Determination

Total count of bacteria (including of Azotobacter genus), actinobacteria, and molds were determined according to the plate method. The groups of microorganisms were cultured on solid substrates using appropriate dilutions of soil solutions, expressed as CFU·g⁻¹ of soil dry matter. Counts of heterotrophic bacteria were determined on the Merck standard agar medium (peptone from casein 5 g·L⁻¹, meat extract 3 g·L⁻¹, NaCl 5 g·L⁻¹, agar 12 g·L⁻¹) following 5 to 6-day incubation at temperature of 28 °C [42].
Numbers of *Azotobacter* sp. were determined by placing 1 g of the soil sample on a Petri dish which was mixed with a medium (KH$_2$PO$_4$ 0.5 g·L$^{-1}$, MgSO$_4$ 0.2 g·L$^{-1}$, NaCl 0.2 g·L$^{-1}$, CaCO$_3$ 5 g·L$^{-1}$, sucrose 10 g·L$^{-1}$, agar 12 g·L$^{-1}$ and traces of Mn, Fe and Mo) according to Döbereiner [43]. Plates were incubated for 5 days at temperature of 24 °C. Numbers of actinobacteria were determined on Pochon selective medium (aspragine 0.05 g·L$^{-1}$, nystatin 0.01 g·L$^{-1}$, K$_2$HPO$_4$ 5 g·L$^{-1}$, MgSO$_4$·7 H$_2$O 2.5 g·L$^{-1}$, NaCl 2.5 g·L$^{-1}$, MnSO$_4$·5 H$_2$O 0.05 g·L$^{-1}$, Fe$_2$(SO$_4$)$_3$·5 H$_2$O 0.05 g·L$^{-1}$, agar 20 g·L$^{-1}$) with starch (2 g·L$^{-1}$) addition [44] incubating plates for 7 days at temperature of 26 °C. The count of molds was determined on a selective medium (KH$_2$PO$_4$ 1 g·L$^{-1}$, MgSO$_4$ 0.5 g·L$^{-1}$, glucose 10 g·L$^{-1}$, agar 20 g·L$^{-1}$) with 3.5 mL·L$^{-1}$ rose bengal agar (Sigma Aldrich, St. Louis, MI, USA) and with 0.1 g·L$^{-1}$ aureomycin added. Plates were incubated for 6 days at temperature of 25 °C [45]. All analyses were replicated 5-fold. In order to determine the state of biological balance of the soil, the microbiological index of soil quality was determined according to Myśkow [46]. The calculation formula is as follows (Equation (1)):

$$X = EU + AC \times MO^{-1}$$

where: EU-Bacteria, AC-Actinobacteria, MO-Molds count.

2.5. Statistical Analyses

The collected data were subjected to a conventional analysis of variance using STATISTICA® 10 (StatSoft, Krakow, Poland). Homogeneous subsets of means were identified with Tukey’s test at a significance level of $p < 0.05$. The Pearson correlation coefficient was used to quantify the strength of the relationships between the number of microorganisms and the contents of soil available nutrients. In the second step, Principal Component Analysis (PCA) was used to illustrate the dependence between the count of microorganisms and soil chemical properties of soil as well as weather conditions.

3. Results

Yields of sward in consecutive years of study were affected by the management system (Table A2). However, significant differences were only recorded in 2007 (+33%) and in 2008 (+22%). As a rule, irrespective of the study year, the highest yield was the attribute of the 1st grazing/mowing cycle. Its share in the total yield, depending on a particular year, varied from about 30% in 2007 and 2008 to 42% in 2006. The sward yields of consecutive grazing cycles were significantly lower, but as a rule significantly higher in the mowing system.

3.1. Microbial Population

The composition of the soil microbial community in the long-term pasture underwent a significant variability in response to two management systems, i.e., grazing and mowing (Table 2, Figure 1). The greatest impact on the microbial composition was exerted by the weather in consecutive years. The coefficient of variation for the microorganisms count increased in the order:

EU (42%) < AC (49%) < AZ (56%) < MO (69%)

In 2006, a strong difference was observed between the soil microbial community composition in their response to the sward management system in consecutive grazing cycles. Only *Azotobacter* count responded significantly to a change in the type of pasture management, decreasing in the mowed sward. This trend underwent changes in consecutive Gr/Mo cycles, showing at the end of the 2006 vegetative growing season a significantly higher population density in soil under mowed sward. This proved to be a dominant trend of *Azotobacter* population response to the management system, in spite of fluctuation, during consecutive years of study.

In spite of a high year-to-year variability, the molds population showed in general, a constant trend in response to the sward management system. In 2006, the first response of molds to the management system was recorded after the 3rd Gr/Mo cycle. The molds count in soil under mowed sward increased
5-fold as compared to the grazed sward. The same trend was observed in other years. The eubacteria population, for most of the studied grazing cycles, irrespectively of the weather course in consecutive years, showed a relatively low sensitivity to the sward management system. A higher bacteria count in soil under grazing was only significant in two of the 12 Gr/Mo cycles. Actinobacteria showed the strongest response to the interaction of all experimental factors (Figure 3). As in the case of bacteria, the maximum count of these microorganisms was recorded after the 3rd Gr/Mo cycle, i.e., in August. The effect of pasture management on the actinobacteria number was the most remarkable in dry years. No significant advantage for either of the management systems was observed for the first Gr/Mo cycle. In dry years, in the full summer and the early autumn, i.e., after the 3rd and 4th Gr/Mo cycle, the mowing system had a significant, even huge impact on the actinobacteria population compared to the grazed system.

Table 2. Microbial population counts in pasture soil under two different management systems and subjected to grazing/mowing cycles, during a three-year period.

| Year | Grazing/Mowing | Management | EU 1 | AC 2 | MO 3 | AZ 4 | X 5 |
|------|----------------|------------|------|------|------|------|-----|
|      | Cycle          | System     | cfu g⁻¹ DM of Soil | Index |
| 2006 | 1              | Gr         | 24.0 a | 11.0 a | 4.9 b,c | 55.5 d,e | 26.3 a |
|      | Mo             | 30.5 a     | 12.7 a | 5.8 c | 24.7 b,c | 32.7 a |
|      | 2              | Gr         | 77.0 b | 63.2 c | 5.5 b,c | 18.7 a,b | 88.7 b |
|      | Mo             | 69.1 b     | 47.1 b | 3.8 b,c | 16.2 a | 82.6 b |
|      | 3              | Gr         | 95.6 c | 56.0 c | 1.6 a | 54.2 d,e | 131.8 c |
|      | Mo             | 77.3 b     | 72.2 d | 8.6 d | 56.7 e | 85.7 b |
|      | 4              | Gr         | 98.3 c | 63.4 c | 1.6 a | 29.7 c | 141.1 c |
|      | Mo             | 138.8 d   | 98.5 e | 3.7 b | 49.1 d | 166.3 d |
| 2007 | 1              | Gr         | 46.2 b | 39.8 c,d | 4.2 b,c | 19.3 b,c | 56.1 a,b |
|      | Mo             | 96.3 d     | 78.3 f | 5.6 c,d | 21.6 c | 110.9 c |
|      | 2              | Gr         | 41.9 a,b | 14.8 a | 2.2 a,b | 39.0 d | 49.6 a |
|      | Mo             | 44.9 a,b | 36.2 c | 8.0 d | 49.5 e | 49.6 a |
|      | 3              | Gr         | 105.0 d | 45.5 d | 1.4 a | 9.0 a | 136.5 d |
|      | Mo             | 61.0 c     | 54.6 e | 7.5 d | 11.6 a,b | 68.5 b |
|      | 4              | Gr         | 33.6 a | 26.9 b | 1.5 a | 13.1 a,b,c | 51.6 a |
|      | Mo             | 41.1 a,b | 34.9 c | 1.9 a,b | 36.8 d | 61.1 a,b |
| 2008 | 1              | Gr         | 46.1 a | 36.5 a | 3.8 a | 16.4 b | 56.7 a |
|      | Mo             | 53.1 a,b | 35.6 a | 3.8 a | 15.1 b | 63.5 a |
|      | 2              | Gr         | 66.9 c | 58.6 c,d | 12.4 c | 39.1 d | 71.6 a,b |
|      | Mo             | 65.3 c     | 49.1 b,c | 7.6 b | 47.1 e | 71.8 a,b |
|      | 3              | Gr         | 86.2 d | 66.5 d | 1.7 a | 65.8 f | 128.0 d |
|      | Mo             | 93.5 d     | 89.8 e | 9.7 b | 25.3 c | 102.8 c |
|      | 4              | Gr         | 54.2 b | 43.9 a,b | 1.8 a | 10.3 a | 81.5 b |
|      | Mo             | 104.0 e | 102.4 f | 14.0 c | 22.0 c | 111.3 c |

ANOVA

| df  | Year | 141.6 *** | 249.3 *** | 77.2 *** | 156.4 *** | 79.5 *** |
|-----|------|-----------|-----------|----------|-----------|--------|
|     | Cutting frequency | 3.566.3 *** | 380.9 *** | 28.7 *** | 90.5 *** | 318.6 *** |
|     | Management system | 90.0 *** | 490.0 *** | 247.6 *** | 0.6 n,s | 0.6 n,s |
|     | Year * Cut | 298.5 *** | 318.0 *** | 33.3 *** | 275.8 | 172.8 *** |
|     | Year * System | 17.5 *** | 20.1 *** | 7.4 ** | 59.9 | 2.8 n,s |
|     | Cut * System | 171.3 *** | 107.6 *** | 75.3 *** | 134.3 | 133.8 *** |
|     | Year * Cut * System | 59.6 *** | 82.9 *** | 48.5 *** | 57.0 | 18.5 *** |

Gr—grazed, Mo—mowed pasture sward; 1 Eubacteria, 2 Actinobacteria, 3 Molds, 4 Azotobacter, 5 Microbiological index; a the same letter indicates a lack of significant differences within the treatment; *** , ** , * indicate significance at p < 0.001, <0.01, and <0.05, respectively.
3.2. Mineral Nitrogen

The content of $N_{\text{min}}$, as the key factor affecting grass sward productivity, showed high sensitivity to the interaction of all the studied factors (Table 3). Distinct differences were recorded between trends of nitrate ($N\text{-NO}_3$) and ammonium ($N\text{-NH}_4$) content variability (Figures 4 and 5). The $N\text{-NO}_3$ content, averaged over years, was the highest in 2008, and the lowest in the wet 2007. An evaluation of the effect of consecutive Gr/Mo cycles on $N_{\text{min}}$ content variability during the growing season requires a detailed analysis, including the impact of the management systems (Figure 4). The lowest content of $N\text{-NO}_3$ was recorded after the first Gr/Mo cycle. On average, the $N\text{-NO}_3$ content decreased up to the 3rd grazing cycle (Figure 4). In dry years, the $N\text{-NO}_3$ content after the 3rd cycle was higher in the soil under mowed sward. After the 4th Gr/Mo cycle, the significant impact of the mowing system was revealed in 2008. A completely different trend was observed in the wet 2007. The highest $N\text{-NO}_3$ content was recorded just after the 1st Gr/Mo cycle. For subsequent cycles, it was low, especially in soil under the grazing system.

The average content of ammonium ($N\text{-NH}_4$), averaged for Gr/Mo cycles and management systems, was much lower compared to the $N\text{-NO}_3$ content (Table 3, Figure 5). The lowest content of $N\text{-NH}_4$, constituting only 17% of $N\text{-NO}_3$, was recorded in 2008. In other years, the content of this N form was only slightly higher. The trend of the $N\text{-NH}_4$ content was highly specific. Its highest content was recorded after the 1st Gr/Mo cycle. In 2006, it was 4-and 9-fold higher with respect to the amount of $N\text{-NH}_4$ recorded in 2007 and 2008. As a rule, it declined in the subsequent two grazing cycles, reaching its lowest value after the 3rd cut. A recovery in the $N\text{-NH}_4$ content was recorded after the 4th Gr/Mo cycle, with the exception of 2006.
Table 3. Soil chemical properties in pasture soil under two different management systems and subjected to grazing/mowing cycles, during a three-year period.

| Year | Grazing/Mowing Cycle | Management | N-NO₃ | N-NH₄ | N₉₉ | P | K | Na | Mg | pH |
|------|----------------------|------------|-------|-------|-----|---|---|---|----|----|
|      |                      | System     | mg kg⁻¹ soil |       |       |     |   |    |    |    |
| 2006 | 1                    | Gr         | 32.3 b  | 76.8 c  | 109.1 d  | 37.1 e  | 141.5 f  | 37.3 h  | 40.5 a  | 7.20 a |
|      | Mo                   | 20.0 a     | 87.4 f  | 107.4 d  | 31.8 d  | 37.1 b  | 45.7 c  | 52.3 d  | 7.26 a |
|      |                      | 35.0 c     | 22.0 b  | 79.0 h  | 27.4 c  | 64.3 h  | 26.0 c  | 44.0 gb  | 7.47 bc |
|      | 3                    | Gr         | 36.3 b  | 32.4 d  | 69.0 b  | 26.2 c  | 27.4 ab  | 25.9 a  | 45.2 b  | 7.46 bc |
|      | Mo                   | 59.6 c     | 17.1 b  | 76.7 h  | 15.2 a  | 59.4 a  | 27.2 a  | 47.4 bc  | 7.54 a |
|      |                      | 76.5 d     | 20.2 bc | 96.7 c  | 14.5 a  | 37.0 b  | 29.2 a  | 57.0 a  | 7.54 a |
|      | 4                    | Gr         | 109.1 d  | 16.8 b  | 124.9 a  | 24.3 b  | 114.6 b  | 59.3 a  | 49.8 ab  | 7.41 b |
|      | Mo                   | 103.4 e    | 11.5 f  | 114.9 f  | 23.0 b  | 24.3 b  | 37.0 a  | 64.1 e  | 7.41 b |

| 2007 | 1                    | Gr         | 62.7 c  | 24.2 b,c  | 86.9 c  | 21.8 d  | 65.5 d  | 25.0 e  | 51.3 b,c  | 7.49 ab |
|      | Mo                   | 77.6 d     | 15.4 a  | 93.0 a  | 20.4 c,d  | 21.6 a  | 24.7 b  | 56.1 d  | 7.47 a |
|      |                      | 28.7 c,d  | 28.3 cd | 57.0 a  | 20.7 d  | 44.4 b  | 24.8 b  | 41.2 a  | 7.56 ab |
|      | 2                    | Gr         | 37.2 b  | 29.4 d  | 66.9 b  | 18.7 bc  | 20.7 a  | 19.2 b  | 44.7 ab  | 7.56 ab |
|      | Mo                   | 39.3 c  | 23.3 b  | 52.8 a  | 17.0 ab  | 53.0 c  | 19.2 a  | 47.4 bc  | 7.59 ab |
|      |                      | 38.6 b  | 27.5 b,c,d | 66.1 b  | 16.5 a  | 17.0 a  | 21.0 a  | 57.0 d,e  | 7.60 b |
|      | 3                    | Gr         | 29.5 c  | 24.0 b,c  | 53.4 a  | 18.5 b,c  | 64.6 d  | 34.4 b  | 55.0 c,d  | 7.54 ab |
|      | Mo                   | 37.2 c  | 47.7 e  | 85.0 a  | 27.0 a  | 18.5 a  | 23.3 bc  | 60.3 b  | 7.51 ab |

2008 | 1                    | Gr         | 65.4 a  | 8.7 b  | 74.2 a  | 22.5 f  | 63.5 ef  | 35.4 ab  | 47.9 a  | 7.40 ab |
|      | Mo                   | 73.1 a     | 9.3 ab  | 82.4 a  | 20.4 e  | 22.5 b  | 36.7 ab  | 57.0 b  | 7.39 a |
|      |                      | 105.4 e  | 15.9 d  | 121.3 c,d | 17.7 d  | 67.4 a  | 34.5 a  | 52.4 ab  | 7.47 ab |
|      | 2                    | Gr         | 92.7 b  | 12.2 b,c  | 104.9 b  | 15.9 e  | 17.7 ab  | 37.9 a  | 53.3 bc  | 7.44 ab |
|      | Mo                   | 89.6 b  | 10.9 ab  | 103.5 b  | 13.4 b  | 57.1 a  | 35.1 ab  | 57.3 a  | 7.50 ab |
|      |                      | 114.4 c  | 12.9 cd  | 127.2 d  | 12.4 b  | 13.4 ab  | 37.4 d  | 62.4 ab  | 7.48 ab |
|      | 3                    | Gr         | 86.8 b  | 29.1 e  | 117.7 c  | 9.4 a  | 116.1 b  | 39.2 a  | 53.3 bc  | 7.51 ab |
|      | Mo                   | 137.3 d  | 29.6 a  | 167.0 b  | 10.0 b  | 9.4 a  | 44.8 a  | 62.2 b  | 7.44 ab |

ANOVA

| Year | Cut | System | Df | Cut | System |
|------|-----|--------|----|-----|--------|
| 2006 | 2   | 3      | 1  | 6   | 3      |
|      | 2608.3 *** | 481.7 *** | 221.0 *** | 630.1 *** | 637.7 *** |
|      | 1029.6 *** | 486.2 *** | 85.8 *** | 1263.6 *** | 78.7 *** |
|      | 1283.1 *** | 318.7 *** | 240.5 *** | 307.8 *** | 48.9 *** |
|      | 1532.7 *** | 1001.5 *** | 237.3 *** | 293.8 *** | 28.4 *** |
|      | 306.4 ***  | 174.5 ***  | 4195.3 *** | 290.8 *** | 82.4 *** |
|      | 685.9 ***  | 287.8 ***  | 1815.6 *** | 68.0 ***  | 72.3 ***  |
|      | 49.4 ***   | 72.2 ***   | 98.6 ***  | 11.6 ***  | 2.8 ***   |
|      | 49.8 ***   | 46.0 ***   | 107.8 *** | 8.26 ***  | 0.37 ***  |

Gr—grazed; Mo—mowed pasture sward; pH—soil reaction; N-NH₄—Ammonium ion; N-NO₃—Nitrate ion; N₉₉—Mineral nitrogen; Mg—Magnesium; P—Phosphorus; K—Potassium; Na—Sodium; * the same letter indicates a lack of significant differences within the treatment; *** indicates significance at p < 0.001, ** at p < 0.01, and * at p < 0.05, respectively.

Figure 4. Effect of the grassland management in consecutive cuts in the growing season on the nitrate content. * The same letter indicates a lack of significant differences within the treatment (p < 0.05).
Figure 4. Effect of the grassland management in consecutive cuts in the growing season on the nitrate content. a The same letter indicates a lack of significant differences within the treatment (p < 0.05).

Figure 5. Effect of the grassland management in consecutive cut on ammonium content. a The same letter indicates a lack of significant differences within the treatment (p < 0.05).

3.3. Soil pH and Available Nutrients

The pH showed very low variability during the study period, being in the alkaline range (Table 3). Its changes during the vegetative growing season were significantly affected by weather. The highest variability of pH, up to 0.4 units (7.2–7.6), was recorded in the very dry 2006, whereas in the other two years, it ranged to over 7.5.

Phosphorus (P) content was very stable both within each vegetative growing season and between years. As a rule, the highest P content occurred during the 1st Gr/Mo cycle, decreasing along the growth season. The content of available P showed a distinct decrease in consecutive years of study. It decreased from about 250 to 150 mg kg\(^{-1}\) in 2006 and 2008, respectively. The main reason for its steep decline was the lack of P fertilizer application. Its only source, but only in the grazing system, was animal excrement. The advantages of grazing over mowing system was significant in five cases, and the opposite trend applied in only one case (4th Gr/Mo cycle in 2007). The relationship between the P content and the microorganisms population was negative, as has been proved for actinobacteria (AC) (Tables A2 and A3).

The impact of grazing on the content of soil available potassium (K) was very stable. The advantage of grazing over mowing ranged from 2-fold to 12-fold after the 4th Gr/Mo cycle in 2008. The variability in sodium (Na) content was much lower. The content of magnesium (Mg) was, in general, high and stable both within each season and between years. As a rule, a higher content of Mg was recorded in soil under mowed sward. The most decided advantage of mowed sward was observed after the 4th Gr/Mo cycle.

3.4. Relationships between Microbes Count with Available Nutrients

In order to evaluate the relationships between counts of tested microbes groups and the content of inorganic nitrogen with respect to the contrastive systems of long-term pasture sward management, a principal component analysis (PCA) was performed. The analysis clearly reveals a distinctive
strength of relationships between the size of each microbes group and the contents of both inorganic N forms in dependence on the management system and weather (Figures 6 and 7).

Figure 6. (a) Distribution of soil microbiological and chemical properties in the grazed sward and environmental conditions in two PCA axes; (b) Distribution of soil microbiological and chemical properties in the moved sward and environmental conditions in two PCA axes. Legend: EU—Eubacteria, MO—Molds, AC—Actinobacteria, AZ—Azotobacter, X index—microbiological index, T—Temperature, pH, N-NH4—Ammonium ion, N-NO3—Nitrate ion, Nmin—Mineral nitrogen, Mg—Magnesium, P—Phosphorus, K—Potassium, Na—Sodium.

Figure 7. The impact of actinobacteria count on the content of ammonium nitrogen in two systems of pasture management. Legend: Gr—grazing system, Mo—mowing system.
For the grazed sward, PC1 was significantly correlated with 8 of 15 analyzed characteristics (Table A2). The highest, and, at the same time, positive coefficients of correlation (for $R^2 > 0.50$) were recorded for EU, AC, X-index, pH and T, and negative coefficient for N-NH$_4$, P (Table 4). PC2 significantly but negatively correlated with N-NO$_3$, and consequently with N$_{\text{min}}$ and Na. PC3 was significantly, but negatively associated with Azotobacter count, and PC4 positively with the molds population.

The eigenvectors for the examined variables were broadly scattered on the two first PC axes. The closest to absolute of 1 were N-NH$_4$ and P. These two variables were negatively correlated with PC1, but significantly correlated with each other (Table A3). The content of N-NH$_4$ was negatively correlated with the actinobacteria count and showed a negative trend with the bacteria count, and the content of N-NO$_3$. The second group of variables, as results from the distance of eigenvectors to the absolute of 1, were the EB, X-index, AC and Mg, and also T (Figure 6a). The location of the eigenfactor for N-NO$_3$, being much closer to the absolute of 0 than of 1, clearly indicates its much lower importance with an explanation of the 1st and 2nd PC axes variation.

For the moved sward, PC1 was significantly correlated with 9 of 15 analyzed soil characteristics (Figure 6b, Table A4). The highest and, at the same time, positive coefficients of correlation were recorded for N-NO$_3$ and AC ($r > 0.9$). The other significant variables showed much lower values of the coefficient of correlation (positive for EU, X-index, N$_{\text{min}}$, Mg, and negative for P and N-NH$_4$ (Table 4). PC2 was significantly, but negatively associated with Na. PC3 was significantly, and positively associated with molds, and PC4 negatively with Azotobacter count.

For the first group of variables, the closest eigenvector to absolute 1 were the X-index, EU, AC, and N-NO$_3$ (Figure 6b). The strongest relationship between this set of variables was recorded for AC and N-NO$_3$ ($r = 0.86 \ast \ast \ast$). A much lower, but significant relationship was found for AC and N-NH$_4$ ($r = -0.59 \ast$) (Table 4). This set of relationships is identical to that as found in soil under grazed sward.

### 4. Discussion

To explain the in-season variability in the microbes population and community composition requires an accurate knowledge of weather conditions during a respective grazing cycle [23,47]. The most spectacular phenomenon, which greatly impacted the composition of the soil microorganisms

For the grazed sward, PC1 was significantly correlated with 8 of 15 analyzed characteristics (Table A2). The highest, and, at the same time, positive coefficients of correlation (for $R^2 > 0.50$) were recorded for EU, AC, X-index, pH and T, and negative coefficient for N-NH$_4$, P (Table 4). PC2 significantly but negatively correlated with N-NO$_3$, and consequently with N$_{\text{min}}$ and Na. PC3 was significantly, but negatively associated with Azotobacter count, and PC4 positively with the molds population.

The eigenvectors for the examined variables were broadly scattered on the two first PC axes. The closest to absolute of 1 were N-NH$_4$ and P. These two variables were negatively correlated with PC1, but significantly correlated with each other (Table A3). The content of N-NH$_4$ was negatively correlated with the actinobacteria count and showed a negative trend with the bacteria count, and the content of N-NO$_3$. The second group of variables, as results from the distance of eigenvectors to the absolute of 1, were the EB, X-index, AC and Mg, and also T (Figure 6a). The location of the eigenfactor for N-NO$_3$, being much closer to the absolute of 0 than of 1, clearly indicates its much lower importance with an explanation of the 1st and 2nd PC axes variation.

For the moved sward, PC1 was significantly correlated with 9 of 15 analyzed soil characteristics (Figure 6b, Table A4). The highest and, at the same time, positive coefficients of correlation were recorded for N-NO$_3$ and AC ($r > 0.9$). The other significant variables showed much lower values of the coefficient of correlation (positive for EU, X-index, N$_{\text{min}}$, Mg, and negative for P and N-NH$_4$ (Table 4). PC2 was significantly, but negatively associated with Na. PC3 was significantly, and positively associated with molds, and PC4 negatively with Azotobacter count.

For the first group of variables, the closest eigenvector to absolute 1 were the X-index, EU, AC, and N-NO$_3$ (Figure 6b). The strongest relationship between this set of variables was recorded for AC and N-NO$_3$ ($r = 0.86 \ast \ast \ast$). A much lower, but significant relationship was found for AC and N-NH$_4$ ($r = -0.59 \ast$) (Table 4). This set of relationships is identical to that as found in soil under grazed sward.

### 4. Discussion

To explain the in-season variability in the microbes population and community composition requires an accurate knowledge of weather conditions during a respective grazing cycle [23,47]. The most spectacular phenomenon, which greatly impacted the composition of the soil microorganisms
community, was drought during the 2nd grazing cycle in 2006. The shortage of water at the onset of summer (June/July) resulted in complete change in the grass species composition of the mowed sward (Table A1). At the start of the experiment, the grazed pasture was dominated by two species, i.e., perennial ryegrass (33%) and common meadow-grass (30%). There was no change due to drought in the grass species composition of the grazed sward. In the mowed sward, the contribution of orchard-grass in this year increased during the period, extending from the 2nd to the 4th Gr/Mo cycle in 2006 from 3.5% to 39%. All eight subsequent grazing cycles increased step-by-step from 40% to 54%. This grass is more tolerant to water stress than other grass species due to a higher photosynthesis rate, and the same time a lower transpiration rates under water stress [48].

This tremendous shift in the grass species composition resulted in an increase in the population of soil microorganisms by 13%, 28%, 86%, and 2% for bacteria, actinobacteria, fungi, and Azotobacter, respectively. This can be explained by significant differences in the impact of the dominant grass species on the propagation of microorganisms present in the root rhizosphere or bulk soil [2,49–51], the most remarkable being the increase of the molds count in response to the expansion of orchard-grass in the mowed sward. This phenomenon can be explained by the drying–rewetting process, which creates subsequently favorable conditions for mold propagation [23,52]. The 2nd responsive microbial group to the increased share of orchard-grass were actinobacteria. The effect was the most pronounced in the grazing cycle directly following drought (3rd cycle in 2006, and 2008). The mowed sward had a significant, even huge impact on the actinobacteria population compared to the grazed system. The observed phenomenon results from the induction by actinobacteria under stress conditions, such as drought, alkaline soil pH, and recalcitrant plant organic matter, of several different survival strategies [53,54]. All these stress factors acted during this study.

The average content of soil ammonium (N-NH$_4$), averaged for grazing cycles and management systems, was much lower compared to the N-NO$_3$ content (Table 4, Figure 6a,b). In the soil/plant system, it fulfills dual functions, i.e., being a basic substrate for the nitrifying organisms, and a nitrogen source for plant growth [19]. In our study, the in-season trend of the N-NH$_4$ content was highly specific. Its highest content was recorded after the 1st grazing cycle. It then declined, reaching its lowest value after the 3rd grazing cycle. A N-NH$_4$ content recovery was, in general, recorded after the 4th grazing cycle. The trend obtained, in spite of year-to-year variability, is typical for natural grassland soils [53]. The impact of microbial community, i.e., eubacteria and actinobacteria, on the in-season trend of the N-NH$_4$ content was almost the same. However, the way of pasture sward management significantly impacted the N-NH$_4$ release from the soil resources. The N-NH$_4$ content in both management systems responded to the actinobacteria population in the same manner, i.e., fitting the quadrate regression model. The grazing system created more favorable conditions for N-NH$_4$ release from soil resources, as indicated by cardinal indices of the regression models developed (Figure 7). For the grazed sward, the optimum actinobacteria count of 53.6 cfu·10$^4$ resulted in the N-NH$_4$ content of 15.7 mg kg$^{-1}$ soil. For the mowing system, the 74.4 cfu·10$^4$ resulted in the N-NH$_4$ content of 11.0 mg kg$^{-1}$ soil. These two sets of data suggest a higher efficiency of actinobacteria in the grazed sward. This finding can be explained by their capiotrophy, resulting in a high potential to utilize animal excrement [49].

Grazing system: \[\text{N-NH}_4 = 0.018A_c^2 - 2.76A_c + 114.1 \text{ for } n = 12, R^2 = 0.63, p \leq 0.01\] (2)

Mowing system: \[\text{N-NH}_4 = 0.0096A_c^2 - 1.99A_c + 114.1 \text{ for } n = 12, R^2 = 0.56, p \leq 0.01\] (3)

The optimum bacteria count for the minimum N-NH$_4$ content was 76.7cfu·10$^4$ and 103.6cfu·10$^4$, whereas the minimum N-NH$_4$ content was 8.3 and 8.2 kg ha$^{-1}$, for the grazing and mowing systems, respectively. The recovery of the N-NH$_4$ content after the 4th cut content is a result of the decreased competition for nitrogen between plants and microbes. In autumn, the rate of the pasture sward growth lessens, but the rate of ammonium release is still high [53]. The presented models explicitly corroborate the higher potential of actinobacteria as compared to bacteria to release N-NH$_4$ from recalcitrant organic matter [54,55].
The positive relationship between P, K and N-NH$_4$, taking into account the lack of P fertilizer application, indicates P soil content as the key factor limiting inorganic N release from soil resources (Figure 6a,b). This relationship was significant, however, only for the mowing system:

$$P = -0.34AC + 118 \text{ for } R^2 = 0.74, n = 12, P \leq 0.01 \quad (4)$$

The model obtained clearly shows that the increasing population of actinobacteria in the soil under the moved sward resulted in the decreasing content of available P. The presented analysis showed that the effective P-fertility milieu for actinobacteria growth was soil poor in available P but at the same being within the alkaline range. The data obtained fully corroborates the hypothesis of Mander et al. [56] about the much stronger P solubility in response to bacteria action in soil with a low P pool. It has recently been documented that a high soil pH, concomitant with water stress, accelerates the rate of actinobacteria sporulation [57]. A study by Hashimoto et al. [58] showed that Ca$^{2+}$ ions present in the growth milieu of Streptomyces significantly enhance the stimulation of aerial mycelium. These two facts can explain the positive impact of alkaline pasture soil on actinobacteria activity with respect to inorganic N release from organic soil. The advantage of actinobacteria over eubacteria as a microbial agent responsible for inorganic N release from recalcitrant organic matter is a consequence of the response of this group to unfavorable weather conditions [50]. The data obtained explicitly corroborate the higher potential of actinobacteria as compared to bacteria to release N-NH$_4$ from recalcitrant organic matter [54].

The contents of both N forms were negatively correlated with each other, which was significant in the mowing system (Table 4, Table A4, Figure 6a,b). The lowest content of N-NO$_3$, except 2007, was recorded after the first cut. On average, the N-NO$_3$ content increased after the 3rd and especially the 4th cut (Figure 4). The same trend was observed for microbe count (Table 3). In the spring, nitrogen is effectively taken up by the fast-growing grasses [51]. As a consequence, a temporary shortage of available N can significantly decrease the rate of soil microorganisms propagation [25,52]. A shortage of N-NO$_3$ was found to be the key factor limiting the population of microorganisms, except Azotobacter (Table A4). The order of limitation based on the coefficient of correlation was as follows:

Molds < Eubacteria < Actinobacteria.

The population of actinobacteria displayed the most resistance to the shortage of N-NO$_3$. The advantage of this phylum over others can be explained by the ability of these microorganisms to use N from recalcitrant organic matter [55]. The strength of this relationship was much more significant in the moving system (Figure 8). The same type of trend was recorded for bacteria, but their impact on the N-NO$_3$ content was slightly weaker as presented by the regression models developed:

Grazing system: N-NO$_3$ = $-0.026CFU^2 + 3.75CFU - 57.1$ for $n = 12, R^2 = 0.34, p \leq 0.05 \quad (5)$

Mowing system: N-NO$_3$ = $0.93CFU + 2.59$ for $n = 12, R^2 = 0.60, p \leq 0.01 \quad (6)$

The first equation clearly indicates that the N-NO$_3$ content increased up to the bacteria count of 72.1 cfu $10^4$. The higher density of bacteria led to a decrease in N-NO$_3$ content. The course of the model developed corroborates the hypothesis by Schimel and Bennett [19] about the significant impact of the bacteria population on the soil N pool. A quite different model was obtained for the mowing systems. The N-NO$_3$ content increased in accordance with the increasing density of bacteria. The models obtained unambiguously show that the mowing system created more favorable conditions for nitrate release from soil resources.
The study showed that grass yield of the mowed sward was significantly higher as compared to the net yield obtained on the grazed sward. There were, however, no differences in the total biomass (net sward yield on the grazed sward + sward litter) between treatments, irrespective of its management. The mowed sward was not enriched in nutrients by cows during the grazing cycle. However, it was potentially rich in both easily mineralized N and C of microbial origin [32]. Consequently, the harvested yield depended only on the fertilizer N and its soil resources, left by animals before the management system change from grazing into mowing. However, past N resources were sufficiently high because, in autumn 2008, as shown in Figure 4, the N-NO$_3$ content significantly exceeded that recorded in the grazed sward. The key reason for this relationship was actinobacteria activity which resulted in a strong release of N-NH$_4$ from its organic pools (Figure 7a). As recently documented by Zhou et al. [59], a chronic N supply to a permanent grassland results in an enhanced activity of actinobacteria, which leads to an enhanced rate of ammonia nitrification. The stronger impact of actinobacteria on the mineral N content can be also explained by a favorable pH, which has been latterly considered as the key driver of their activity [60]. The favorable growth milieu for actinobacteria, concomitant with their capiotrophy, and a high potential for ammonia oxidation resulted in a higher nitrate content in soil under the mowed sward as compared to the grazed one. The size of N mineral pools in 2008 indicates that the mowing system could be even prolonged for the next year, at least.

5. Conclusions

The 3-year studies showed the microbial community structure to be highly sensitive to the system of long-term pasture management. Grazing affected negatively as compared to mowing the number of fungi, actinobacteria, and bacteria. The content of inorganic N decreased during the growing season, reaching its lowest value after the 3rd Gr/Mo cycle. The impact of microorganisms on the release of inorganic N from soil resources increased in the order: Molds < Bacteria < Actinobacteria. The observed regularity in actinobacteria impacting the inorganic N pools, irrespective of the sward
management system, clearly stresses their importance for N transformation in muck soil. The negative correlation between the content of N-NO$_3$ and the contents of N-NH$_4$ and P clearly indicates that any increase in the first reduced the content of the remaining ones.

The advantage of actinobacteria over other groups was due to their better adaptation to moisture stress, and soil P availability, subsequently leading to a higher release of inorganic N from its organic soil pool. The mowing system of pasture management created much more favorable conditions for both actinobacteria propagation and growth and consequently resulted in a higher amount of released inorganic N. The key reason for the actinobacteria increase in the soil under mowed sward was water stress in summer 2006, which radically changed the grass species composition of sward. The increasing share of orchard-grass in the mowed sward created favorable conditions for actinobacteria growth, which significantly impacted soil mineral N release from its resources in organic soils. It can be finally concluded that the temporally introduced mowing system is an effective way to reach sustainability in long-term pasture management.

**Author Contributions:** Conceptualization, M.M. and J.K.; methodology, A.W.-M., M.M., and W.G.; software, R.Ł., A.W.-M., and W.G.; formal analysis, M.M., J.K., and W.G.; investigation, M.M., J.K., W.G., A.W.-M., and R.Ł.; writing—original draft preparation, M.M. and J.K.; writing—review and editing, M.M. and J.K.; visualization, W.G. and A.W.-M.; supervision, W.G.; funding acquisition, R.Ł. All authors have read and agreed to the published version of the manuscript.

**Funding:** This publication was co-financed within the framework of Ministry of Science and Higher Education programme as Regional Initiative Excellence™ in years 2019–2022. Project No. 005/RID/2018/19.

**Conflicts of Interest:** The authors declare no conflict of interest.
Appendix A

Table A1. The in-season variability of main grass species in the pasture sward in consecutive years of study in dependence on the management system.

| Species            | Year | 2006 | 2007 | 2008 |
|--------------------|------|------|------|------|
|                    |      | 1    | 2    | 3    | 4    |
|                    |      | 1    | 2    | 3    | 4    |
|                    |      | 1    | 2    | 3    | 4    |
|                    |      | 1    | 2    | 3    | 4    |
| Lolium perenne L.  | Grazed | 32.8 ± 2.5 | 29.8 ± 3.2 | 14.4 ± 3.4 | 28.8 ± 3.1 | 25.8 ± 2.1 | 31.5 ± 4.2 | 30.3 ± 3.5 | 34.7 ± 4.1 | 29.1 ± 2.4 | 26.5 ± 2.2 | 24.8 ± 1.8 |
| Poa pratensis L.   | Grazed | 29.5 ± 3.1 | 28.8 ± 3.1 | 13.4 ± 2.8 | 30.5 ± 2.9 | 28.8 ± 2.7 | 24.3 ± 1.6 | 26.8 ± 1.7 | 27.7 ± 2.2 | 26.1 ± 3.1 | 27.3 ± 3.1 | 31.3 ± 3.7 | 33.2 ± 3.1 |
| Dactylis glomerata L. | Grazed | 0.8 ± 0.01 | 1.8 ± 0.02 | 4.6 ± 1.2 | 6.3 ± 0.2 | 9.8 ± 0.3 | 12.2 ± 0.4 | 11.3 ± 0.4 | 7.3 ± 0.2 | 4.3 ± 0.1 | 6.1 ± 0.2 | 6.0 ± 0.2 | 5.8 ± 0.3 |
|                   | Mowed | 32.8 ± 1.5 | 29.8 ± 2.5 | 6.8 ± 2.4 | 12.8 ± 1.3 | 13.3 ± 1.1 | 12.5 ± 0.9 | 13.5 ± 1.1 | 14.5 ± 1.5 | 13.5 ± 1.6 | 13.1 ± 1.4 | 12.3 ± 1.3 | 12.1 ± 1.1 |
| Poa pratensis L.   | Mowed | 29.5 ± 4.1 | 28.8 ± 4.4 | 5.8 ± 3.2 | 12.8 ± 1.3 | 12.8 ± 1.4 | 12.5 ± 1.5 | 13.5 ± 1.5 | 15.1 ± 1.6 | 15.8 ± 1.3 | 15.5 ± 1.8 | 16.5 ± 1.6 | 18 ± 1.1 |
| Dactylis glomerata L. | Mowed | 1.5 ± 0.05 | 3.5 ± 0.04 | 16.9 ± 4.8 | 39.3 ± 4.2 | 42.5 ± 4.4 | 47.1 ± 5.0 | 49.8 ± 3.8 | 50.0 ± 2.2 | 49.8 ± 8.8 | 51.5 ± 2.2 | 53.2 ± 3.4 | 54.3 ± 1.8 |

Table A2. Yields of grass sward in the consecutive grazing/mowing cycles, t ha⁻¹ DM.

| Year | Management System | Grazing/Mowing Cycle | total |
|------|-------------------|----------------------|-------|
|      |                   | 1                    | 2     | 3     | 4     |       |
| 2006 | Grazing           | 2.444 a               | 1.433 a | 0.494 a | 1.228 b | 5.599 a |
|      | Grass litter      | 0.907 ± 0.074         | 0.384 ± 0.048 | 0.199 ± 0.019 | 0.254 ± 0.039 | 1.744 ± 0.180 |
|      | Mowing            | 2.547 b               | 1.765 b | 0.598 b | 0.912 a | 5.822 b |
| 2007 | Grazing           | 2.037 a               | 1.506 a | 1.406 a | 1.168 a | 6.117 a |
|      | Grass litter      | 0.382 ± 0.054         | 0.334 ± 0.036 | 0.328 ± 0.029 | 0.180 ± 0.022 | 1.224 ± 0.141 |
|      | Mowing            | 2.340 b               | 2.043 b | 2.111 b | 1.671 b | 8.165 b |
| 2008 | Grazing           | 2.028 a               | 1.428 a | 1.451 a | 1.372 a | 6.279 a |
|      | Grass litter      | 0.706 ± 0.077         | 0.303 ± 0.042 | 0.263 ± 0.034 | 0.230 ± 0.024 | 1.502 ± 0.177 |

a-b the same letter indicates a lack of significant differences within the treatment and grazing/mowing cycle.
Agronomy 2020, 10, 724

Table A3. Pearson correlation matrix between microbiological and chemical characteristics of pasture soil under grazed system, n = 12.

| Characteristics | AC $^2$ | MO $^3$ | AZ $^4$ | X index $^5$ | N-NO$_3$ | N-NH$_4$ | N$_{\text{min}}$ | P | K | Na | Mg | pH | T $^6$ | P $^7$ |
|-----------------|---------|---------|---------|-------------|----------|----------|-------------|---|---|----|----|----|-------|-------|
| EU $^1$         | 0.81 ** | -0.17   | 0.08    | 0.97 ***    | 0.34     | -0.53    | 0.04        | -0.37 | -0.27 | 0.04 | 0.19 | 0.41 | 0.71 * | 0.02 |
| AC $^2$         | 1.00    | 0.13    | 0.08    | 0.78 **     | 0.63 *   | -0.67 *  | 0.28        | -0.40 | -0.19 | 0.21 | 0.49 | 0.28 | 0.52   | -0.28 |
| MO $^3$         | 1.00    | 0.11    | -0.35   | 0.27        | 0.05     | 0.33     | 0.24        | 0.03  | -0.04 | -0.06 | -0.29 | 0.04 | -0.39 |
| AZ $^4$         | 1.00    | 0.11    | 0.18    | 0.21        | 0.34     | 0.15     | 0.06        | 0.11  | -0.09 | -0.32 | 0.39 | 0.32 |
| X index $^5$    | 1.00    | 0.37    | -0.52   | 0.08        | -0.45    | -0.19    | 0.15        | 0.36  | 0.42  | 0.64 * | 0.06 |
| N-NO$_3$        | 1.00    | 0.43    | -0.69 * | 0.13        | -0.067   | -0.38    | 0.21        | 0.29  | -0.50 | -0.23 | -0.19 |
| N-NH$_4$        | 1.00    | 0.65 *  | 0.66 *  | 0.008       | -0.55    | -0.66 *  | -0.47       | -0.14 |
| N$_{\text{min}}$ | 1.00    | 0.68 *  | 0.72 ** | 0.29        | -0.50    | -0.77 ** | -0.59 *     | -0.34 |
| P               | 1.00    | 0.43    | 0.13    | -0.69 *     | 0.13     | -0.78 ** | -0.36       | -0.26 |
| K               | 1.00    | 0.67 *  | -0.04   | 0.76 **     | -0.27    | 0.47      | -0.27       | -0.15 |
| Na              | 1.00    | 0.25    | -0.47   | 0.27        | -0.18    | -0.44    | 0.21        | 0.19  |
| Mg              | 1.00    | 0.36    | 0.01    | -0.27       | 0.21     | 0.19      | 0.19        | 0.34  |
| pH              | 1.00    | 0.49    | 0.35    | 0.38        | 0.38     | 0.48      | 0.38        | 0.48  |

***, **, * indicate significance at p < 0.001, <0.01, and <0.05, respectively; $^1$ Eubacteria, $^2$ Actinobacteria, $^3$ Molds, $^4$ Azotobacter, $^5$ Microbiological index, $^6$ temperature, $^7$ precipitation; pH, N-NH$_4$—Ammonium ion, N-NO$_3$—Nitrate ion, N$_{\text{min}}$—Mineral nitrogen, Mg—Magnesium, P—Phosphorus, K—Potassium, Na—Sodium.

Table A4. Pearson correlation matrix between microbiological and chemical characteristics of pasture soil under mowed system, n = 12.

| Characteristics | AC $^2$ | MO $^3$ | AZ $^4$ | X index $^5$ | N-NO$_3$ | N-NH$_4$ | N$_{\text{min}}$ | P | K | Na | Mg | pH | T $^6$ | P $^7$ |
|-----------------|---------|---------|---------|-------------|----------|----------|-------------|---|---|----|----|----|-------|-------|
| EU $^1$         | 0.94 ***| 0.25    | 0.14    | 0.97 ***    | 0.54     | -0.59 *  | 0.04        | -0.42 | -0.27 | 0.23 | 0.59 * | 0.02 | -0.01 | -0.03 |
| AC $^2$         | 1.00    | 0.49    | 0.10    | 0.86 ***    | 0.64 *   | -0.66 *  | 0.19        | 0.21  | 0.49 | 0.28 | 0.52 | 0.07 | 0.06  |
| MO $^3$         | 1.00    | 0.02    | 0.11    | 0.57        | 0.63 *   | -0.80 ** | 0.33        | 0.21  | 0.19 | 0.19 | 0.34 |
| AZ $^4$         | 1.00    | 0.15    | 0.12    | -0.15       | 0.04     | -0.10    | 0.27        | -0.07 | 0.02 | 0.17 | 0.38 | 0.48 |
| X index $^5$    | 1.00    | 0.72 ** | -0.56 * | 0.47        | -0.32    | -0.25    | 0.17        | 0.56 * | 0.02 | -0.04 | -0.08 |
| N-NO$_3$        | 1.00    | 0.62 *  | 0.81 ** | -0.73 **    | -0.52    | 0.51     | 0.69 *      | -0.04 | 0.01 | 0.04 |
| N-NH$_4$        | 1.00    | -0.03   | 0.65 *  | 0.43        | 0.17     | -0.29    | -0.44       | -0.37 | -0.12 |
| N$_{\text{min}}$ | 1.00    | -0.44   | 0.79 ** | 0.66 *      | -0.38    | -0.26    | -0.12       | 0.00  |
| K               | 1.00    | 0.02    | -0.39   | 0.35        | 0.07     | 0.07     | -0.29       | -0.29 |
| Na              | 1.00    | 0.04    | -0.79 **| -0.29       | -0.19    | -0.19    | -0.19       | 0.42  |
| Mg              | 1.00    | 0.07    | -0.13   | -0.13       | -0.13    | -0.13    | -0.13       | 0.29  |
| pH              | 1.00    | 0.56    | 0.42    | 0.42        | -0.29    | -0.29    | -0.29       | 0.29  |

***, **, * indicate significance at p < 0.001, <0.01, and <0.05, respectively; $^1$ Eubacteria, $^2$ Actinobacteria, $^3$ Molds, $^4$ Azotobacter, $^5$ Microbiological index, $^6$ temperature, $^7$ precipitation; pH, N-NH$_4$—Ammonium ion, N-NO$_3$—Nitrate ion, N$_{\text{min}}$—Mineral nitrogen, Mg—Magnesium, P—Phosphorus, K—Potassium, Na—Sodium.
References

1. Russel, J.R.; Bisinger, J. Forages and pastures symposium: Improving soil health and productivity on grasslands using managed grazing of livestock. *J. Anim. Sci.* 2015, 93, 2626–2640. [CrossRef]

2. Hale, M.; Deák, B.; Posselt, P.; Valkó, O.; Westerberg, L.; Milberg, P. Grazing vs. mowing: A meta-analysis of biodiversity benefits for grassland management. *Agric. Ecosys. Environ.* 2016, 222, 200–212. [CrossRef]

3. Finneran, E.; Crosson, P.; O’Kiely, P.; Shalloo, L.; Forristal, P.D.; Wallace, M. Economic modelling of an integrated grazed and conserved perennial ryegrass forage production system. *Grass For. Sci.* 2012, 67, 162–176. [CrossRef]

4. Neufeld, K.R.; Garyston, S.J.; Bitman, S.; Krzie, M.; Hunt, D.E.; Smukler, S.M. Long-term alternative dairy manure management approaches enhance microbial biomass and activity in perennial forage grass. *Biol. Fertil. Soils* 2017, 53, 613–626. [CrossRef]

5. Jankowska-Huflejt, H.; Wróbel, T.; Twardy, S. Current role of grassland in development of agriculture and rural areas—An example of mountain voivodship Malopolskie and Podkarpackie. *J. Water Land Dec.* 2011, 15, 3–18. [CrossRef]

6. Warda, M.; Kozlowski, S. Grassland—A Polish resource. *Grass. Sci. Europe* 2012, 17, 3–16.

7. Wasilewski, Z. The efficiency of dairy cow grazing in a large farm. *Water Environ. Rural Areas.* 2011, 2, 173–180.

8. Egan, G.; Zhou, X.; Wang, D.; Jia, Z.; Crawley, M.J.; Fornara, D. Long-term effect of grasslands management on soil microbial abundance: Implications for soil carbon and nitrogen storage. *Biogeochemistry* 2018, 141, 213–228. [CrossRef]

9. Averill, C.; Waring, B. Nitrogen limitation of decomposition and decay: How can it occur. *Glob. Chang. Biol.* 2018, 24, 1417–1427. [CrossRef]

10. Jnawali, A.D.; Ojha, R.B.; Marahatta, S. Role of *Azotobacter* in soil fertility and sustainability—A Review. *Adv. Plants Agric. Res.* 2015, 2, 1–5.

11. Sridhar, B.S. Review: Nitrogen fixing microorganisms. *Int. J. Microbial Res.* 2012, 3, 46–52.

12. Bhatti, A.A.; Haq, S.; Bhat, R.A. *Actinomycetes* benefaction role in soil and plant health. *Microb. Pathog.* 2017, 111, 458–467. [CrossRef] [PubMed]

13. Wolkańska, A.; Gorniak, D.; Zielenkiewicz, U.; Kuzniar, A.; Izak, D.; Banach, A.; Blaszczzyk, M. Actinobacteria structure in autogenic, hydrogenic and lithogenic cultivated and non-cultivated soils: A culture-independent approach. *Agronomy* 2019, 9, 598. [CrossRef]

14. Lenart-Boroń, A.; Banach, T. Soil actinobacteria from *Streptomyces* genus in environment contaminated of heavy metals. *Kosmos* 2014, 1, 87–93.

15. Brookes, C.; Báath, E. Fungal and bacterial growth responses to N fertilization and pH in the 150-year “Park Graa” UK grassland experiment. *Microbiol. Ecol.* 2011, 76, 89–99.

16. Arcand, M.M.; Helgason, B.L.; Lemke, R.L. Microbial crop residue decomposition dynamics in organic and conventionally managed soils. *Appl. Soil Ecol.* 2016, 107, 347–359. [CrossRef]

17. Hartman, W.H.; Richardson, C.J. Differential nutrient limitation of soil microbial biomass and metabolic quotients (qCO2): Is there a biological stoichiometry of soil microbes? *PLoS ONE* 2013, 8, e57127. [CrossRef]

18. Frac, M.; Hannula, S.E.; Belka, M.; Jedrzejka, M. Fungal biodiversity and their role in soil health. *Front. Microbiol.* 2018, 9, 707. [CrossRef]

19. Schimel, J.P.; Bennett, J. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology* 2004, 85, 591–602. [CrossRef]

20. Riggs, E.; Hobbie, S.E. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. *Soil Biol. Biochem.* 2016, 99, 54–65. [CrossRef]

21. Zhao, F.; Ren Ch; Shelton, R.S.; Wanf, Z.; Pang, G.; Chen, J.; Wang, J. Grazing intensity influence soil microbial communities and their implications for soil respiration. *Agric. Ecosys. Environ.* 2018, 249, 50–56. [CrossRef]

22. Olivera, N.L.; Prieto, L.; Bertillier, M.; Ferrero, M.A. Sheep grazing and soil bacteria diversity in shrublands of the Patagonian Monte, Argentina. *J. Arid Environ.* 2016, 125, 16–20. [CrossRef]

23. Hammerl, V.; Kastl, E.-M.; Schloter, M.; Kublik, S.; Schmidt, H.; Welzl, G.; Jentsch A; Beerthuelsen, C.; Gschwendtner, S. Influence of rewetting on microbial communities involved in nitrification and denitrification in a grassland soil after a prolonged drought period. *Sci. Rep.* 2019, 9, 2280. [CrossRef] [PubMed]
24. Geisseler, D.; Lazicki, P.A.; Cow, K.M. Mineral nitrogen input decreases microbial biomass in soil under grasslands but not annual crops. *Appl. Ecol.* 2016, 106, 1–10. [CrossRef]
25. Cui, J.; Holden, N.M. The relationship between soil microbial activity and microbial biomass, soil structure and grassland management. *Soil Till. Res.* 2015, 146, 32–38. [CrossRef]
26. Yang, F.; Niu, K.; Collind, C.G.; Yan, X.; Ji, Y.; Ling, N.; Zhou, X.; Du, G.; Guo, H.; Hu, S. Grazing practices affect the soil microbial community composition in a Tibetan alpine meadow. *Land. Degrad. Dev.* 2019, 30, 49–59. [CrossRef]
27. Pan, Y.; Cassman, N.; De Hollander, M.; Mendes, L.W.; Korevaar, H.; Geerts, R.H.E.M.; Van Veen, J.A.; Kuramae, E.E. Impact of long-term N, P, K and NPK fertilization on the composition and potential functions of the bacterial community in grassland soil. *FEMS Microbiol. Ecol.* 2014, 90, 195–205. [CrossRef]
28. Wakelin, S.; Mander, C.; Gerad, E.; Jabsa, J.; Erb, A.; Young, S.; Condron, L.; O’callaghan, M. Response of microbial communities to contrasted histories of phosphorus fertilization in pastures. *Appl. Soil Ecol.* 2012, 61, 40–48. [CrossRef]
29. Kristensen, T.; Søegaard, K.; Kristensen, I.S. Management of grasslands in intensive dairy livestock farming. *Livest. Prod. Sci.* 2005, 96, 61–73. [CrossRef]
30. Belanger, G.; Rochette, P.; Chantigny, M.; Ziadi, N.; Angers, D.; Charbonneau, É.; Pellerin, D.; Liang, C. Nitrogen availability from dairy cow dung and urine applied to forage grasses in eastern Canada. *Can. J. Plant Sci.* 2015, 95, 55–65. [CrossRef]
31. Semmartin, M.; Garibaldi, L.A.; Chanet, E.J. Grazing history effects on above-and below-ground litter decomposition and nutrient cycling in two co-occurring grasses. *Plant Soil* 2008, 303, 177–189. [CrossRef]
32. Linag, C.; Schimel, J.P.; Jastrow, J.D. The importance of anabolism in microbial control over soil carbon storage. *Nat. Microbiol.* 2017, 2, 1–6. [CrossRef]
33. Fornara, D.A.; Tilman, D.; Hobbie, S.E. Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. *J. Ecol.* 2009, 97, 48–56. [CrossRef]
34. Van Beek, C.L.; Van den Eertwegh, G.A.P.H.; Van Schaik, F.H.; Velthof, G.L.; Oenema, O. The contribution of dairy farming on peat soil to N and P loading of surface water. *Nutr. Cycl. Agroecosyst.* 2004, 70, 85–95. [CrossRef]
35. Taube, F.; Giers, M.; Hermann, A.; Loges, R.; Schönbach, P. Grassland and globalization—challenges for north-west European grass and forage research. *Grass Forage Sci.* 2014, 69, 2–16. [CrossRef]
36. Grzyb, S.; Pronczuk, J. Division and Valorisation of Meadow Habitats and Evaluation of Their Production Potential. In Proceedings of the National Meadow Conference SGGW, Warsaw, Poland, 27–28 September 1994; pp. 51–63.
37. Jongman, R.H.G.; Bunce, R.G.H.; Metzger, M.J.; Mucher, C.A.; Howard, D.C.; Mateus, V.L. Objectives and applications of a statistical environmental stratification of Europe. *Landsc. Ecol.* 2006, 21, 409–429. [CrossRef]
38. PN-EN ISO 13395: 2001. *Water Quality—Determination of Nitrite and Nitrate Nitrogen and Their Sum by Flow Analysis (CFA and FIA) with Spectrophotometric Detection*; ISO: Geneva, Switzerland, 2001.
39. Sapek, A.; Sapek, B.; Vymazal, J. Methods in Applied Soil Microbiology and Biochemistry; Academic Press: London, UK, 1995; pp. 51–141.
40. Grzyb, S.; Kuremis, B. The colorimetric determination of phosphorus with ammonium vanadate-molybdate and its application in plant analysis. *J. Plant Nutr. Soil Sci.* 1952, 159, 11–21.
41. PN-EN ISO/IEC 17025: 2018-02. *General Requirements for the Competence of Testing and Calibration Laboratories*; ISO: Geneva, Switzerland, 2018.
42. Merck-Polska. 101621 Standard Count Agar for Microbiology. 2004, p. 1. Available online: https://www.merckmillipore.com/PL/pl/product/Standard-count-agar,MDA_CHEM-101621#anchor_TI (accessed on 10 November 2016).
43. Döbereiner, J. *Isolation and Identification of Aerobic Nitrogen-Fixing Bacteria from Soil and Plants*; Eds.; Nannipieri, P., Eds.; Methods in Applied Soil Microbiology and Biochemistry; Academic Press: London, UK, 1995; pp. 134–141.
44. Kariska, Z.; Grabirińska-Łoniewska, A.; Lebkowska, M.; Żechowska, E. *Laboratory Exercises in Sanitary Biology*; Warsaw Polytechnic: Warsaw, Poland, 2001; p. 193.
45. Albaum, S.; Masaphy, S. Comparison of rose bengal-chloramphenicol and modified aureomycin-rose bengal-glucose-peptone agar as media for the enumeration of molds and yeasts in water by membrane filtration techniques. *J. Microb. Method.* 2009, 76, 310–312. [CrossRef]
46. Myśków, W. An attempt to use microbial activity indicators to assess soil fertility. *Microb. Adv.* 1981, 20, 173–192.

47. Frey, S.D.; Lee, J.; Melillo, J.M.; Six, J. The temperature response of soil microbial efficiency and its feedback to climate. *Nat. Clim. Chang.*, 2013, 3, 395–398. [CrossRef]

48. Staniek, M.; Kocóń, A. Forage grasses under drought stress in conditions of Poland. *Acta Physiol. Plant.* 2015, 37, 116. [CrossRef]

49. Carey, J.; Beman, J.M.; Eviner, V.T.; Malmstrom, C.M.; Hart, S.C. Soil microbial community structure is unaltered by plant invasion, vegetation clipping, and nitrogen fertilization in experimental semi-arid grasslands. *Front. Microbiol.* 2015, 6, 466. [CrossRef]

50. Naylor, D.; Coleman-Derr, D. Drought stress and root-associated bacterial communities. *Front. Plant Sci.* 2018, 8, 2223. [CrossRef]

51. Bardgett, R.D.; Hobbs, P.J.; Frostegård, Å. Changes in soil fungal: Bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biol. Fert. Soils* 1996, 22, 261–264. [CrossRef]

52. Jurburg, S.D.; Natal-Da-Luz, T.; Raimundo, J.; Morais, P.V.; Sousa, J.P.; Van Dirk Van Elsas, J.; Salles, J.F. Bacterial communities in soil become sensitive to drought under intensive grazing. *Sci. Tot. Environ.* 2018, 618, 1638–1646. [CrossRef]

53. Anandan, R.; Dharumadurai, D.; Gopinath, P.M. An Introduction to Actinobacteria. In *Actinobacteria—Basics and Biotechnological Applications*; Dhanasekaran, D., Jiang, Y., Eds.; InTech Publisher: London, UK, 2016.

54. Shivlata, L.; Satyanarayama, T. Thermophilic and alkaliphilic *Actinobacteria*: Biology and potential applications. *Front. Microb.* 2015, 6, 1014. [CrossRef]

55. Alster, C.H.J.; Koyama, A.; Johnson, N.G.; Wallenstein, M.D.; Con Fisher, J.C. Temperature sensitivity of soil microbial communities: An application macromolecular rate theory to microbial respiration. *J. Geophys. Res. Biogeosci.* 2016, 121, 1420–1433. [CrossRef]

56. Mander, C.; Wakelin, S.; Young, S.; Condron, L.; O’callaghan, M. Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. *Soil Biol. Biochem.* 2012, 44, 93–101. [CrossRef]

57. Fang, B.Z.; Salam, N.; Han, M.X.; Jiao, J.Y.; Cheng, J.; Wei, D.Q.; Xiao, M.; Li, W.J. Insights on the Effects of heat pretreatment, pH, and calcium salts on isolation of rare *Actinobacteria* from Karstic Caves. *Front. Microbiol.* 2017, 8, 1535. [CrossRef]

58. Hashimoto, M.; Kondo, T.; Kozone, I.; Kawaide, H.; Abe, H.; Natsume, M. Relationship between response to and production of the aerial mycelium-inducing substances *Pamamycin*-607 and A-factor. *Biosci. Biotechnol. Biochem.* 2003, 67, 803–808. [CrossRef]

59. Zhou, X.; Fornara, D.; Wasson, E.A.; Wang, D.; Ren, G.; Christie, P.; Jia, Z. Effects of 44 years of chronic nitrogen fertilization on the soil nitrifying community of permanent grassland. *Soil Biol. Biochem.* 2015, 91, 76–83. [CrossRef]

60. Jenkins, S.N.; Waite, I.S.; Blackburn, A.; Husband, R.; Rushton, S.P.; Manning, D.C.; O’Donnell, A.G. Actinobacterial community dynamics in long term managed grasslands. *Anton. Leeuwe.* 2009, 95, 319–334. [CrossRef] [PubMed]