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Comparison of Culturable Microbial Groups Present in Selected Peatlands in Midlands of the Republic of Ireland: Effect of 'Peatland Use' Type on Microbial Consortia; A Pilot Study

Gouri Atapattu
School of Food Science & Environmental Health

Samuel Apori
School of Food Science & Environmental Health

Tara Battersby

See next page for additional authors

Article

Effect of 'Peatland-Use' Type on Culturable Microbial Groups in Irish Peatlands in the Midlands

Gouri Atapattu [1,2], Samuel Apori [1,2], Tara Battersby [2], Michelle Giltrap [1,3] and Furong Tian [1,3,*]

[1] School of Food Science & Environmental Health, City Campus, Technological University Dublin, Grangegorman, D07ADY7 Dublin, Ireland; d21125206@mytudublin.ie (G.A.); d21125192@mytudublin.ie (S.A.); michelle.giltrap@tudublin.ie (M.G.)
[2] Environmental Sustainability & Health Institute, City Campus, Technological University Dublin, Grangegorman, D07ADY7 Dublin, Ireland; eshi@tudublin.ie
[3] Nano lab Research Centre, FOCAS Research Institute, City Campus, Technological University Dublin, Camden Row, D08CKP1 Dublin, Ireland
* Correspondence: furong.tian@tudublin.ie

Abstract: Soil microbial ecology in the Irish wetlands is still poorly understood, although it is crucial in introducing effective rewetting schemes to restore and conserve the Irish peatlands. As an initiative, peatlands with distinct land-use types (cutaway, raised semi-degraded, unimproved grassland and grassland) were collected from farms in the midlands to analyse various microbial populations. Peat was homogenized and serially diluted to culture on a range of specific and non-specific culture media. Culture isolation and microbial enumeration were performed. Gram staining and other microscopic observations of morphologically distinct microorganisms were performed, followed by isolation procedures. The numbers of total viable bacteria of cutaway bog and unimproved grassland were 4.23×10^3 CFU g^{-1} and 9.81×10^7 CFU g^{-1}, respectively, with a significant statistical difference ($p \leq 0.05$). Raised semi-degraded bogs comprised low values of both aerobes and fungal populations. *Penicillium* spp. and *Trichoderma* spp. were common in many vegetation types. Phosphate-solubilizing bacteria were present in the majority of the study sites. This indicated that the soluble form of phosphorus was being assimilated by plants. Cutaway peat contained the bacteria with the highest phosphate-solubilizing index (3.167). Overall, the number of culturable microbial groups in cutaway and raised semi-degraded peatlands exhibited significant differences, while the rest did not show drastic changes according to land-use type. This study provides baseline data to continue studies on bog microbiology, which provides a new outlook for restoration. Future work should consider microbial interaction with environmental variables in different land-use types.

Keywords: aerobes; fungi; anaerobes; phosphate solubilization; raised bog; grassland

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1. Introduction

Irish peatlands cover about 20% of the land surface across the Republic of Ireland [1]. Peatlands are a class of wetlands [2] that act as a massive pool of sequestered carbon [3]. Peat formation in these lands is an important ecological process that mitigates the effects of climate change and minimizes greenhouse gas (GHG) emissions. Energy production, horticultural production [4] and aesthetic purposes [5] are a few remarkable benefits of these lands. Unfortunately, due to land use for peat extraction, agricultural practices and forest management [6], a significant proportion of the peat area (approximately 80%) has been damaged. The remainder (peatlands in a natural state) are subjected to slow degradation [7]. Natural peatlands can mostly extract carbon from the atmosphere and pool it within peat [8]. Therefore, implementing proper rewetting schemes to restore degraded peatland ecosystems [9] has received much attention. A recent study from Germany

showed the risk of grassland renewal on GHG emissions (about 9–35% of European peatlands are grasslands). The study targeted a drained grassland. The study concluded that factors such as mechanical grassland renewal, water level increase and a lack of grass cover lead to severe GHG emissions [10].

Rewetting every drained peatland is challenging as it requires a proper understanding of the terrestrial carbon cycle, the plant community and the soil microbial community. Moreover, the soil microbial community greatly impacts the carbon cycle since part of carbon sequestration is carried out by it [3]. Although several research studies have been conducted in the context of Irish peatlands [9,11–13], investigating the role of microorganisms present in Irish peat has not been carried out more broadly. One such infrequent study was based on the western Atlantic coast of Ireland [14]. The study explored the biomass of different types of decomposers and consumers in three differently vegetated peatlands. Nevertheless, only viable bacteria and fungi were studied under microflora in that particular study. A recent study conducted on a montane blanket bog illustrated the effects of restoration on bacterial community structure in two seasons. The season appeared to be influential on bacterial community structure [15]. However, a descriptive image of the microbial groups could have been portrayed. Recently, the impact of water table level and the addition of fertilizers on microbial community structure was investigated on a drained peatland in Ireland. However, it was noted that the microbial communities remained unchanged against the treatments [16]. The presence of nitrogen-fixing bacteria in peat has been also focused on in the literature [17]. Both the grassland and peatland tested in that study contained only anaerobic nitrogen-fixing bacteria, indicating that anaerobic organisms in peat are crucial in the nitrogen cycle. In addition, the vegetation type of peat has an undeniable influence on the bacterial and fungal communities in peat. In a recent review, the unique characteristics of *Sphagnum* mosses, which promote the growth of endophytic bacteria and fungi, were demonstrated [18]. The importance of understanding the peat microbiome turned out to be a key emphasis of that study.

Apart from Ireland, previous research about peatland microbial communities in the world was principally based on methanogenic organisms [19]. Other groups of microorganisms in the peat ecosystem were not targeted in the research in a wider context. Some microbiology-based studies on peat have been carried out in the United States, Canada, Thailand and Australia [17–20]. It is still at a rudimentary level in Ireland. One of our recent pilot studies was conducted to collect preliminary data on distinct microbial groups present in Irish bogs in the midlands. The study analyzed whether microbial groups change according to temporal variation. The data collected from that study give valuable future directions that will pave the way to the establishment of promising rewetting schemes to reduce greenhouse gas emissions [21]. There is a paucity of soil microbiology-based studies in Irish peatlands. Microbes in drained peatlands might differ from their pristine conditions (the pristine condition of a peatland in its original state often refers to a 'near-natural' condition). This finding also came out from a peatland study in Australia where they discovered that fungal and prokaryotic richness was greatly reduced after peatland degradation [20]. It is considered that degraded peatlands reach 'near-pristine conditions' through restoration. Monitoring restoration success is important to assess how effective existing restoration practices (e.g., rewetting) are. The data obtained for different 'land-use' types can be employed as bio-indicators to monitor the progress of restoration in the near future. Currently, the availability of microbial data in Western Europe is inadequate [22]. Considering this knowledge gap, this pilot study was initiated to fulfil the following aims and objectives: (i) to collect baseline data on culturable microbial populations across four 'peatland-use' types; (ii) to make comparisons between the microbial numbers in each site; and (iii) to demonstrate the future possibility of microbial communities to act as an environmental measure for peatland restoration.

Our study hypothesized that microbial numbers and communities could differ according to the land-use types and pH. Therefore, culture isolation as the conventional method was employed in the study to find bio-indicator organisms. Finding bio-indicator

organisms is a long-term goal. It involves screening existing or new microbial strains. Isolation of prominent categories like total viable bacteria, aerobic bacteria and fungi is crucial for a basic understanding of an ecological niche. Specific groups like fungi, anaerobes and actinomycetes can be important in terms of finding bio-indicators. Phosphate-solubilizing bacteria imply the possibility of bio-fertilizers in land applications. Also, these groups were not studied extensively under bog microbiology. Our study initiated the first step that is required for this process through culture isolation. Although the current study collected data on six types of microbial groups across four different land-use types (grassland, unimproved grassland, cutaway bogs and raised semi-degraded bogs), this is the first time this type of pilot microbiological study has been conducted in the Republic of Ireland. Grasslands are considered to be a vital part of pasture-based systems in Ireland [12]. A characteristic grass cover and other plant species can be observed in grasslands. Unimproved grassland is another basic category of grasslands. But, they are only subjected to traditional land-management practices or ploughing. Artificial fertilizers were not added to these lands [23]. Cutaway peatlands are lands used for mechanical peat harvesting. As a result, the vegetation is destroyed in cutaway sites. Prior to harvesting, they are classified as bogs [24]. Raised semi-degraded bogs are concentrated in the midlands. This habitat type can be restored to 'active raised bogs' via proper land-management practices. Bog moss layers, such as *Sphagnum* species, are prominent in these lands [25].

2. Materials and Methods

2.1. Sample Sites and 'Peatland-Use' Types

The study sites were located in the midlands (Figure 1a) of the Republic of Ireland, specifically in (i) County Offaly (53°9′46″ N 7°39′10″ W) and (ii) County Laois (53°9′49″ N 7°37′18″ W). Average monthly precipitation in Co. Offaly and Co. Laois are approximately 65 mm and 68 mm, respectively. Peat across four 'peatland-use' types was collected, including cutaway rough grazing, raised semi-degraded bogs, grassland (Figure 1b) and unimproved grassland (Figure 1c) sites. All the samples were collected on 14 April 2022.

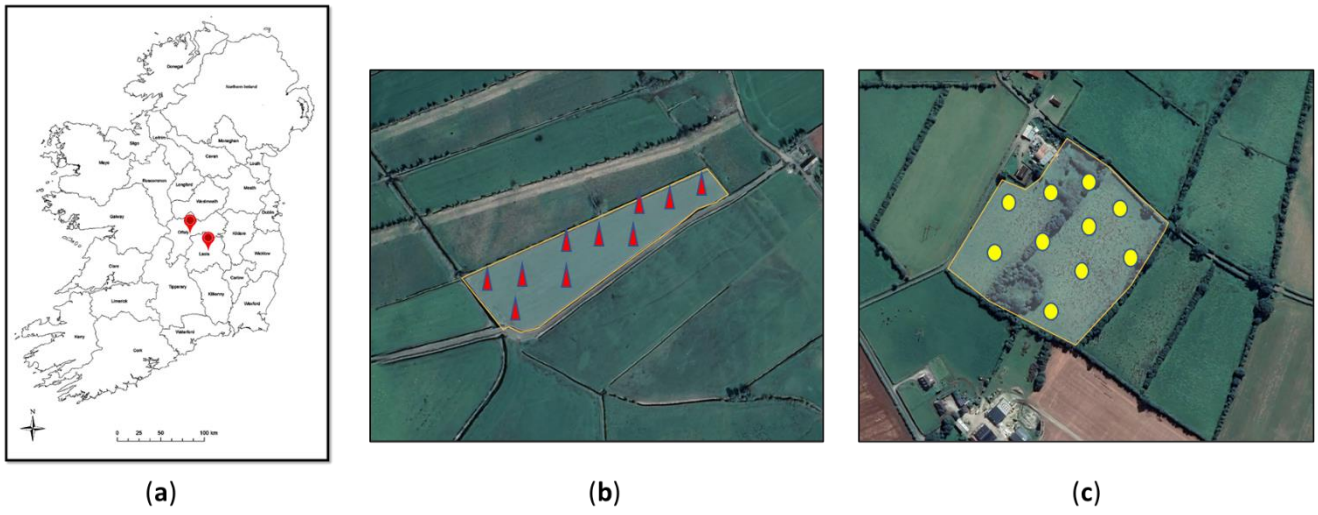

Figure 1. (**a**) Map of the Republic of Ireland indicating the sampling venues in the midlands; (**b**) the satellite view of the grassland site located in Co. Laois. Sampling points (n = 10) are illustrated in red triangles; (**c**) the satellite view of unimproved grassland site located in County Offaly. Sampling points (n = 10) are illustrated in yellow circles.

2.2. Sample Collection

The 'peatland-use' type was taken into consideration during the sample collection. Each site was divided in the form of zigzags to collect samples. A composite sample was made from each site by collecting ten replicate samples (n = 10) from each site. The purpose

of replicating the sample points was to minimize the errors in sample collection. Peat soil was collected between the depth ranged from 0 to 15 cm (0 cm refers to the surface of the peat). The relevant soil depth was measured, and peat was dug using a standard soil knife. Approximately 250–300 g of peat was collected and packed into sterile airtight containers. Samples were transported to the laboratory and kept in refrigerator at 4–6 °C.

2.3. Isolation of Distinct Groups of Culturable Microorganisms

2.3.1. Isolation and Enumeration of the Total Viable Bacteria (TVB) and Aerobic Bacteria (AB)

Isolation and enumeration of TVB and AB was carried out according to the method described by [21] Gouri et al. (2023). Composition of culture media was described in the supplementary material. Small stones, fauna and plant debris in peat samples were removed by sieving through a pore size of 2 mm. Fresh peat (10.0 g) was suspended in 90.0 mL of sterile maximum recovery diluent. It was dispersed in a homogenizer at 140 rpm for 1 h. A total of 1 mL of initial suspension was serially diluted by 10-fold stages. The serial dilutions 10^{-1}–10^{-7} were pour-plated (1 mL aliquots) in triplicate on nutrient agar for the enumeration of the total viable bacteria. The pH of the medium was 7.0 ± 0.2. Likewise, the serial dilutions were used to perform the spread plate (0.1 mL aliquots) technique on nutrient agar (Condalab, Madrid, Spain) and tryptic soy agar (TSA) (Condalab, Madrid, Spain) plates for the detection of aerobic bacteria. The plates were incubated at 37 ± 1 °C in an inverted position for 48–72 h. Colony counts were reported after the incubation period. This procedure was repeated on peat soil from the four different sites separately. Bacterial numbers in Log CFU g^{-1} (CFU = colony forming units) of AB and TVB were used to produce the ratio of AB vs. TVB in each site. The most accurate dilution was taken for colony counting. Microbial culturing was performed in triplicate, and triplicate colony counts were obtained at each case. Microbial data were reported as CFU g^{-1}. They were converted into logarithmic form and used for the statistical analysis in triplicate values.

2.3.2. Isolation and Enumeration of the Culturable Fungal Population

Serial dilutions (10^{-1}–10^{-7}), performed as described in Section 2.3.1., were pour-plated on a half-strength Czapek–Dox (Condalab, Madrid, Spain) agar medium. Filter-sterilized streptomycin (50 μg/mL) was added to the agar to inhibit the bacterial growth. The plates were incubated for 2–3 weeks at 25 ± 1 °C, and colony counts were reported. Single fungal colonies with different morphologies were subcultured (transferring a colony or cells to fresh media to purify) on new half-strength Czapek–Dox agar media by dissecting each fungal colony with a sterile scalpel and forceps. In parallel, fungal slides were prepared to observe fungal spores, conidia, hyphae and sporangia under the light microscope. Fungal isolates were primarily identified according to the descriptions and mycological keys by Gams and Bissett (1998) and the *Textbook of Fungi* [26,27]. This method was performed on peat from four land-use types separately.

2.3.3. Isolation and enumeration of Anaerobic Bacteria

The serial dilutions (10^{-1}–10^{-7}), as described in Section 2.3.1., were used to carry out the pour-plate technique on anaerobic agar (Condalab, Madrid, Spain). The plates were incubated at 25 ± 1 °C for 2–3 weeks under anaerobic conditions. An anaerobic jar was used for this purpose. Microbiology Anaerocult® A, a reagent for the creation of an anaerobic medium, was kept inside the anaerobic jar. The colony counts were reported following the incubation period. To obtain pure cultures, morphologically distinct colonies were chosen and streaked three consecutive times. Gram-negative and Gram-positive bacteria were differentiated by Gram staining. This procedure was repeated on peat soil samples from all the study sites separately.

2.3.4. Isolation of Phosphate-Solubilizing Bacteria (PSB)

Pikovskaya medium was prepared according to the method described by [21]. Pikovskaya agar was initially prepared without adding dextrose (glucose) to avoid sugar caramelization. Dextrose solution was sterilized using a 0.45 μm filter membrane and added to the sterile Pikovskaya agar separately. The phosphate substrate concentration was 5.0 g/L, maintained at pH 7. The serial dilutions (10^{-1}–10^{-7}) as described in Section 2.3.1. were spread (0.1 mL) on Pikovskaya agar. The control was set up by spreading 0.1 mL of sterile maximum recovery diluent onto Pikovskaya agar. The plates were incubated at 30 ± 1 °C for three weeks. Colonies with halozones were detected. Halozones are the 'halo' or clear zone around the colony due to the phosphate solubilization of bacteria. The diameters were measured using a standard ruler. The reading error for a standard ruler with mm increments was +/−0.1 mm under optimal conditions. The uncertainty value was indicated for the diameter lengths in centimetres [28]. The following equation was used to compute the phosphate solubilization index (PSI) for each colony:

Phosphate-solubilising index (PSI) = {(Diameter of the halozone + colony) cm/(Diameter of the colony) cm}

2.3.5. Isolation of Actinomycetes

Serial dilutions (10^{-1}–10^{-7}), as described in Section 2.3.1., were used to carry out the pour-plate technique on starch casein agar (SCA). The plates were incubated at 25 ± 1 °C for 2–3 weeks. After the incubation period, morphologically different colonies were picked and streaked (subculture) on sterile nutrient agar plates. Three successive streak isolations were performed to obtain pure actinomycete cultures. Plates that contained halozones were treated with an iodine solution to observe whether starch hydrolyzed. In parallel, Gram staining was performed for the purified isolates.

2.3.6. Glycerol Stock Preparation

The purpose of preparing glycerol stocks was to preserve the isolated microbial cultures for further studies. A stock solution (200 mL) of glycerol (40% *v*/*v*) was prepared by mixing glycerol (80 mL) with de-ionized water (120 mL). The stock solution was autoclaved. Pure culture isolates from bacterial groups (total viable bacteria, aerobic bacteria, anaerobes, phosphate-solubilising bacteria and actinomycetes) were inoculated in nutrient broth separately by transferring a single colony to the medium. Fungal colonies were inoculated in Czapek–Dox modified broth. Broth cultures were incubated overnight. Overnight pure culture (500 μL) was aseptically banked in a sterilized 40% (*v*/*v*) glycerol solution (500 μL) in sterile cryovials. Cryovials were gently mixed and labeled. They were stored at −80 °C.

2.4. Measuring the Soil pH

The pH in each study site was measured using the soil survey standard test method. The pH meter was calibrated prior to obtaining the readings. A total of 10.0 g of peat was weighed into a clean Duran bottle and 50 mL of de-ionized water was added into it to prepare a 1:5 soil: water suspension (*w*:*v*). The soil suspension was mechanically shaken for 1 h at 15 rpm (LABWIT, ZWYR-D2402, Shanghai, China). The electrode was immersed into the soil suspension, and triplicate measurements of pH were recorded.

2.5. Statistical Analysis

Prism version 9.4.0 Graph pad Software, Inc. was used to produce the graphs and the statistical analysis. Samples in each land-use type were separately analyzed in three independent replicates. Triplicate values were converted to logarithmic form. They were used for the statistical analysis. Microbial numbers were verified for normality by Kolmogorov–Smirnov and Shapiro–Wilk tests ($p < 0.05$). The data were checked for homoscedasticity by Levene's test ($p < 0.05$). Due to non-homogeneous variances in data, differences between microbial numbers were analyzed by the Kruskal–Wallis H test ($p < 0.05$). Peat

microbial numbers were converted to logarithmic base 10. Error bars of all figures were presented using the mean with standard deviation (SD). Multiple-comparison analysis was performed by Dunn's multiple-comparisons test unless otherwise stated. Group comparisons between total viable bacteria and aerobic bacteria in four study sites were performed by the Friedman test.

3. Results

3.1. Total Viable Bacterial (TVB) Counts across Vegetation Patterns

Figure 2a shows the population of total viable bacteria in four types of peat soil, including cutaway bogs, unimproved grassland, raised semi-degraded and grassland. Microbial numbers of total viable bacteria were the greatest in unimproved grassland (9.81×10^7 CFU g^{-1}) compared to the rest, while cutaway peat recorded the lowest quantity of total viable bacterial population (4.23×10^3 CFU g^{-1}) (Figure 2a). The number (9.81×10^7 CFU g^{-1}) was also higher by four magnitudes than the cutaway land. There is a statistically significant difference between the total viable bacterial quantity between the unimproved grassland and cutaway site ($p < 0.0001$). But the difference in microbial number between other sites were not significant ($p \geq 0.05$) (Figure 2a). Overall, it was evident that there was not a significant difference in the microbial content across the four study sites, except for the cutaway bog and the unimproved grassland. According to Table 1, the temperatures of each land-use type ranged from 6–7 °C. Cutaway (5.35 ± 0.04 pH) and raised semi-degraded bog (4.52 ± 0.1 pH) were comparatively more acidic than the unimproved grassland (6.19 ± 0.07 pH) and grassland (6.08 ± 0.06 pH).

Table 1. Physiological factors of each 'land-use' type on the date of collection.

Land-Use Types	Average pH of the Peat Soil	Temperature (°C)
Cutaway	5.35 ± 0.04	6
Unimproved grassland	6.19 ± 0.07	7
Raised semi-degraded	4.52 ± 0.1	6
Grassland	6.08 ± 0.06	7

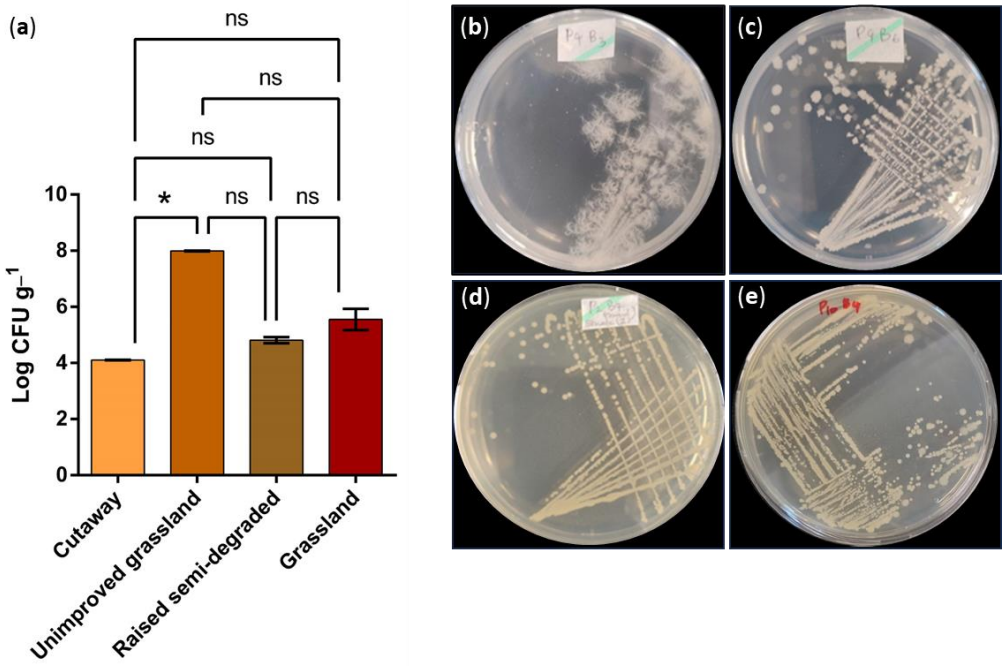

Figure 2. (**a**) Number of total viable bacteria across four study sites. ns, not significant ($p > 0.05$); * $p \leq 0.05$; (**b**) typical viable bacterial pure culture isolated from the cutaway bog; (**c**) typical viable

bacterial pure culture isolated from the raised semi-degraded bog; (**d**) aerobic bacterial isolate grown on nutrient agar from unimproved grassland sample; (**e**) aerobic bacterial culture isolated from raised semi-degraded bog.

3.2. Comparison of TVB and Aerobic Bacterial (AB) Counts and the AB/TVB Ratio

Aerobic bacterial counts were obtained from the spread plate technique. Figure 3a shows that cutaway bog represented the highest number (1.53×10^6 CFU g^{-1}) of aerobic bacterial count than the rest, although it contained the lowest total viable bacteria. Group comparisons of the Friedman test revealed that differences between TVB and AB counts in the four study sites were not very prominent ($p \geq 0.05$), as shown in Figure 3a. However, aerobic bacterial counts between the cutaway bog and the raised semi-degraded bog were significantly different ($p \leq 0.05$). Overall, AB typically ranged from 3.81×10^4 to 1.53×10^6 CFU g^{-1}, with the smallest number given from raised semi-degraded bog. The ratio of AB/TVB was greater than 1 in both cutaway bogs and grassland sites (Figure 3b). This could be an indication of higher proportions of aerobes present in these sites. Cutaway had the highest ratio of AB/TVB, and unimproved grassland demonstrated the lowest ratio.

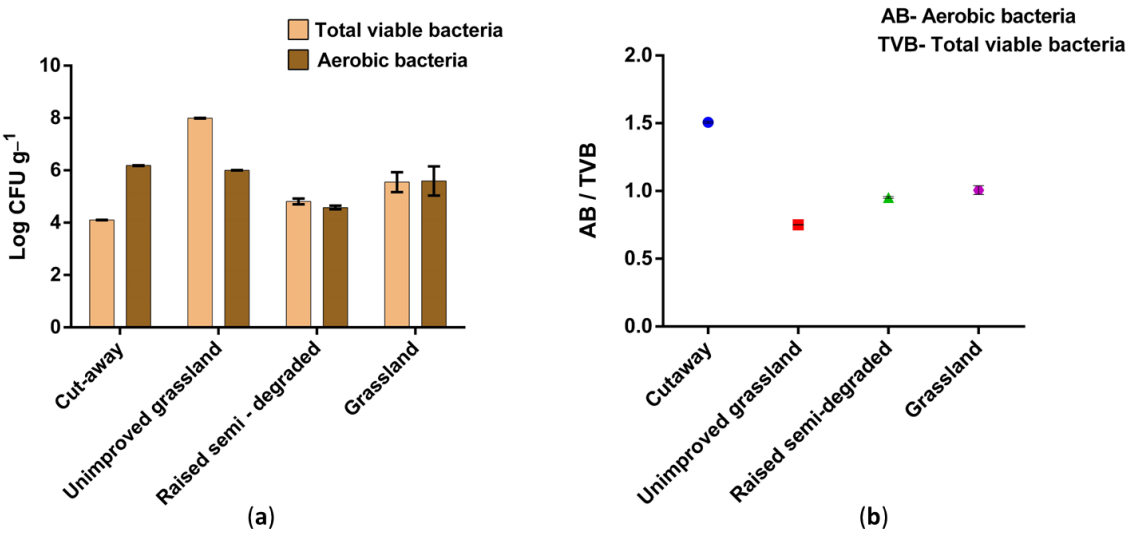

Figure 3. (**a**) Comparison of total viable bacteria and aerobic bacteria; (**b**) the ratio of AB to TVB in each peatland site.

3.3. Morphology of Bacteria under Light Microscopy

Pure bacterial isolates examined under the light microscope revealed that most of the isolates were primarily Gram-positive rods (Figure 4a). This could be an indication that *Bacillus* spp. endospore-forming bacteria were detected: (i) cells with central endospores and (ii) cells with sub-terminal endospores. The proportion of Gram-negative (Figure 4b) isolates was comparatively less than that of Gram-positive bacteria. Occasional Gram-positive cocci cells were detected. This observation was common in all the land-use types tested in this study.

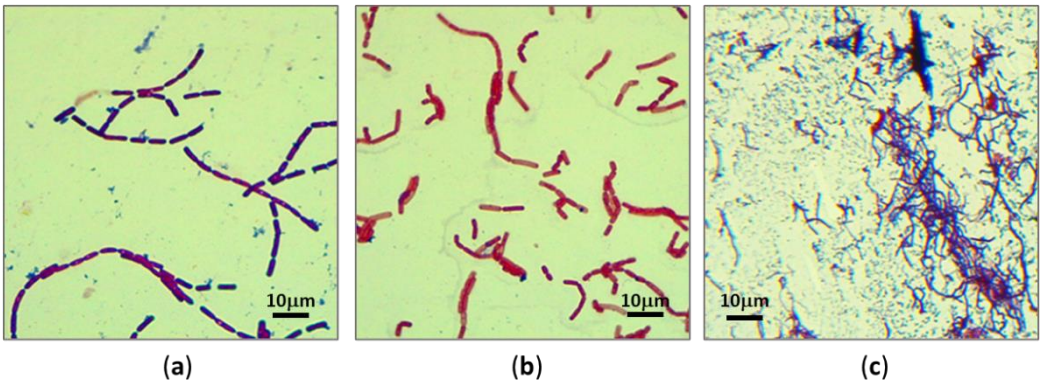

Figure 4. Bacterial pure cultures examined under an oil immersion lens (10 × 100) of light microscope. (**a**) Gram-positive bacilli; (**b**) Gram-negative rods; (**c**) Gram-positive cells of actinomycete isolate.

3.4. Fungal Populations

The diversity of peat fungi from each study site was high. This was observed from the cultural and morphological evidence, along with the overall fungal population number in each study site. Similar to TVB microbiota, unimproved grassland contained the highest quantity of fungi (4.79×10^4 CFU g^{-1}). In contrast, raised semi-degraded bog showed the lowest fungal numbers (5.83×10^2 CFU g^{-1}). According to Figure 5a, the fungal count in raised semi-degraded bog significantly varied with the unimproved grassland ($p < 0.0001$). The rest of the peatland sites did not show prominent differences in their fungal number ($p \geq 0.05$).

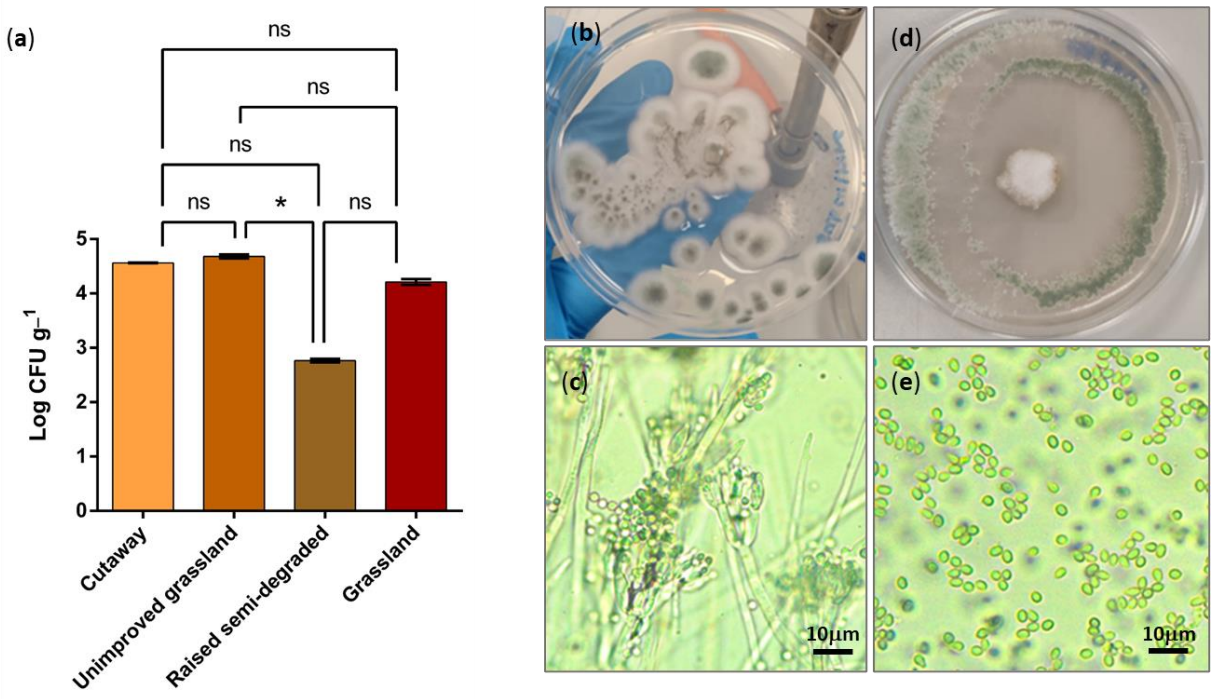

Figure 5. (**a**) Fungal population across four study sites. ns, not significant ($p > 0.05$); * $p \leq 0.05$; (**b**) *Penicillium* spp. colony morphology on Czapex–Dox agar; (**c**) image of *Penicillium* spp. conidia-bearing structures under light microscope (10 × 100); (**d**) *Trichoderma* spp. colony morphology on Czapex–Dox agar; (**e**) image of *Trichoderma* spp. spores under light microscope (10 × 100).

Colonies were observed to grow on the Czapek–Dox agar medium, while some originated from inside, spreading the hyphae towards the surface of the solid medium.

Microscopic observation of fungal hyphae, conidia and characteristic conidia-bearing structures revealed the presence of *Penicillium* (Figure 5b,c), which is an Ascomycete anamorphic fungus. It was detected in all the study sites. Interestingly, microfungi like *Penicillium* spp. had faster growth rates than the other fungal isolates. However, it took approximately 2–3 weeks to dominate the plate by other slow-growing fungal species. Another feature exhibited by some fungal species is the formation of concentric rings when they develop as a colony. This is important in identifying isolates like *Trichoderma* spp. The colony formation and its microscopic appearance of asexual spores are shown in Figure 5d,e. One major observation, conducted through the microscopic details of every fungal isolate, was that the mycelia contained green-color globular cells assembled inside the hyphae. They could be an indication of algal cells migrating inside the fungal hyphae. The association of motile bacteria with fungi was observed in all the isolates. Motility of motile bacteria resembled Brownian movement.

3.5. Enumeration of Anaerobes across the Vegetation Types

The maximum level of depth in peat was 15 cm. Therefore, the quantities of anaerobes described in this case are only valid for the peat above 15 cm of depth. Green-color colonies appeared after three weeks of incubation. Gas accumulation was observed at the bottom of the Petri dishes. Some colonies grew at the bottom of the anaerobic agar layer. Some colonies grew in the middle of the anaerobic agar layer. Few colonies developed on the surface of the agar. The quantities of anaerobic bacteria across four study sites are illustrated in Figure 6a. The grassland sample reported the highest number of anaerobes. The number of anaerobes typically ranged from 2.16×10^4–2.29×10^6 CFU g^{-1}. These values are markedly lower than that of the total viable and aerobic bacterial counts, irrespective of the study site. As illustrated in Figure 6a, there was no significant difference between the cutaway, unimproved grassland and the raised semi-degraded bog ($p > 0.05$), which was dissimilar to the previously elucidated microbial groups. In contrast, anaerobes in grassland were approximately two magnitudes higher than that in the raised semi-degraded sample. The values were statistically significant ($p = 0.0003$). The predominant organisms isolated were Gram-negative short rods. However, Gram-positive rods were arranged in chains. Occasional Gram-positive cocci isolates were detected. Some Gram-positive short rods showed swellings in the middle of the cell. Terminal endospores were observed in rods. This observation was common in the land-use types listed in this study.

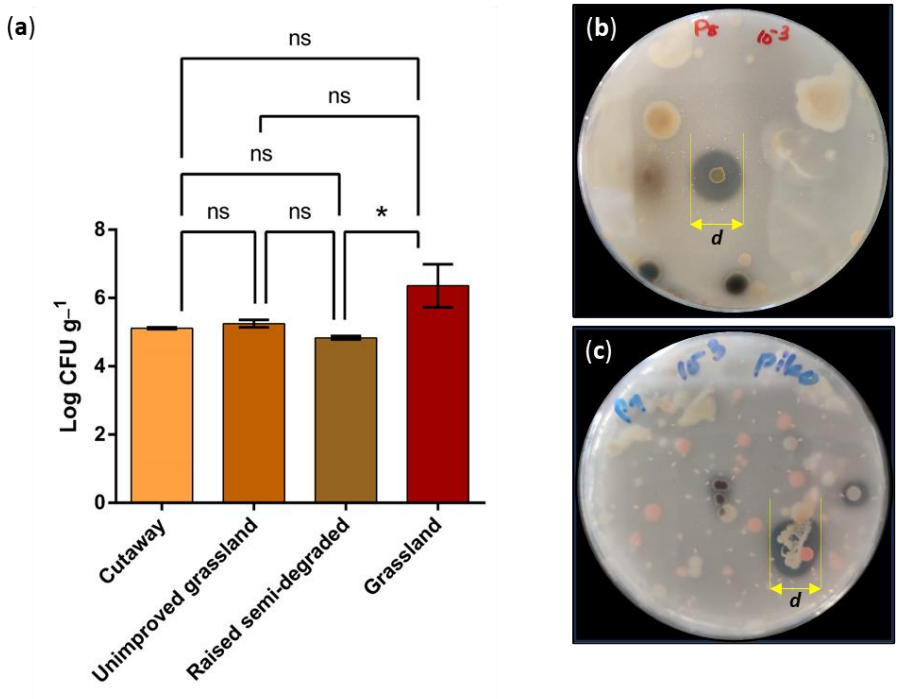

Figure 6. (**a**) Quantity of anaerobic population across four different sites. ns, not significant ($p > 0.05$); * $p \leq 0.05$; (**b**) Halozone developed by PSB isolated from cutaway bog on solid Pikovskaya medium; (**c**) Halozone developed by PSB isolated from grassland on solid Pikovskaya medium; *d* = diameter of the colony and the halozone.

3.6. Phosphate-Solubilizing Bacteria (PSB) and Phosphate-Solubilizing Index (PSI)

Phosphate solubilization was evaluated using the Pikovskaya medium, which contained β-tri-calcium phosphate as the sole source of insoluble, inorganic phosphate. The phosphate concentration and the pH of the medium were 5.0 g/L and pH 7.0, respectively. Therefore, PSI was reported for the conditions at pH 7.0. The diameter of the halozones (Figure 6b,c) increased as the diameter of the colonies increased. The maximum diameter was reached after approximately 2–3 weeks. According to Table 2, PSB exhibiting a remarkable ability to solubilize calcium phosphate was isolated from cutaway peat (SI = 3.167 and 3.000, respectively). However, raised semi-degraded bog was the only site where no PSB was detected. It was noted that approximately 75% of the PSB isolates demonstrated PSI values in the range of 1–2. Only 25% of the total PSB isolates illustrated PSI values above 2. Comparatively, the weakest ability to solubilize the inorganic phosphates was demonstrated by the unimproved grassland (SI = 1.4).

Table 2. Phosphate-solubilizing index of PSB isolates in three land-use types.

Study Site	Average Diameter of the Colony (cm)	Average Diameter of the Colony + Halozone (cm)	Phosphate Solubilization Index (PSI)
Cutaway	0.5 ± 0.1	1.5 ± 0.1	3.000
Cutaway	0.6 ± 0.1	1.9 ± 0.1	3.167
Cutaway	1.25 ± 0.1	2 ± 0.1	1.6
Unimproved	0.5 ± 0.1	0.7 ± 0.1	1.4
Unimproved	0.55 ± 0.1	0.95 ± 0.1	1.727
Grassland	1.1 ± 0.1	1.55 ± 0.1	1.409
Grassland	0.5 ± 0.1	0.8 ± 0.1	1.600
Grassland	0.4 ± 0.1	0.65 ± 0.1	1.625

3.7. Detection and Isolation of Actinomycetes

Colony growth on SCA was observed after 2–3 weeks. Clear zones around the colonies were detected. The clear zones remained colorless after the addition of iodine solution (Figure 7a). The presence of substrate mycelium (Figure 7b) and aerial mycelium was observed in some isolates. Some colonies were distinct from typical bacterial colonies in terms of color, texture, shape and elevation. They appeared as 'pellet'-like structures on the surface of the agar. Figure 7b–f shows the pigmented (pink, grey, brown, maroon, yellow and orange) isolates. Only a few colonies resembled typical bacterial colonies, with a slimy and smooth texture. Gram-positive and clusters of branched filamentous bacteria were detected. They resembled the microscopic view of filamentous fungi.

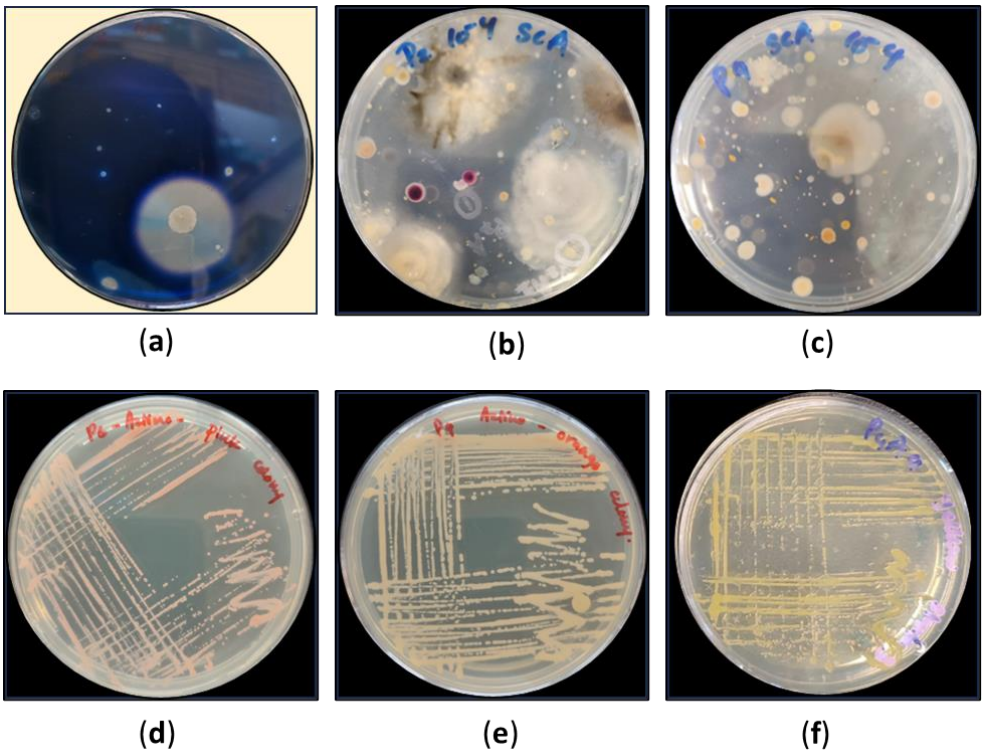

Figure 7. (**a**) Clear zone around a possible actinomycete colony following the iodine treatment; (**b**) development of substrate mycelium on SCA and pigmented colonies (maroon); (**c**) development of pigmented colonies (yellow) on SCA agar; (**d**) streak isolation of a pigmented (pink) culture isolated from unimproved grassland; (**e**) streak isolation of a pigmented (orange) culture isolated from cutaway site; (**f**) streak isolation of a pigmented (yellow) culture isolated from grassland site.

4. Discussion

The diversity and function of microorganisms in peatlands is a vast topic to address. Soil heterogeneity could be one of the many factors creating this enormous variation in peat microbiota. In this study, six distinct microbial groups present in Irish peatlands have been explored regarding their population, characteristics and land-use type. The sites sampled were primarily classified as northern wetlands. It should be emphasized that microbial cultivation techniques in general cannot be expected to enumerate unculturable cells in environmental samples. Therefore, this study solely represented a proportion of culturable microbial populations and their elemental characteristics. The presented microbial numbers were strongly dependent on the culture conditions, such as temperature, pH and nutrients in culture media. Therefore, laboratory culture conditions employed in this study could limit the isolation of culturable bacteria in peat samples. Microbial numbers of total viable bacteria ranged from 1.26×10^4 CFU g^{-1} to 9.81×10^7 CFU g^{-1} among the four sites (Figure 2a). This was consistent with the previous literature where the total direct bacterial counts are approximately 100-fold higher than the culturable bacteria due to laboratory culture media [29].

Overall, the highest typical viable bacterial concentration was recorded in the unimproved grassland sample (9.81×10^7 CFU g^{-1}). This result is in line with similar studies carried out previously. Grayston and co-authors identified that the vegetation type could greatly govern the soil microbial communities present in upland grasslands [30]. Since unimproved grasslands are partially categorized as areas that have not been heavily fertilized, their microbial population could be naturally higher than the other grassland types, which was also seen in our study. Physiological factors, such as temperature and pH of the peat soil, are important parameters that affect the microbial number. According to Table 1, the pH of unimproved grassland (6.19 ± 0.07) was much closer to the neutral

condition of the agar medium. This could be a reason for the higher numbers of total viable bacteria and fungi detected in the site. A slight pH difference in the peat soil and the culture medium could increase the chance of microbial adaptation to artificial culture media. Cutaway bog demonstrated a significantly lower total viable bacterial count (Figure 2a) than the rest. The literature describes how cutaway bogs form when the peat has been removed over several years. Therefore, the uniformity of the peat is disrupted, leading to extensive changes in the soil's physiological and chemical properties [31]. Therefore, colonization of cutaway bogs by microbes is not a rapid process. This can also be another reason for the lower bacterial counts reported from cutaway than the rest in our study. Raised semi-degraded bog was more acidic (4.52 ± 0.1) than all the study sites mentioned here. Acidopyllic microorganisms can thrive well under acidic conditions. But when they were inoculated in a more neutral culture medium, growth might be restricted to a certain extent. The bacterial and fungal numbers of the raised semi-degraded bog were comparatively lower than the rest of the sites. Another factor which could result in lower bacterial numbers is the drawdown of the water table. It has the same effect on bacterial diversity [32].

Little is known about fungal populations and their diversity across peatlands. Similar to bacterial biomass and diversity, it is dependent upon the vegetation/land and the nutrient availability of the peatland [3]. As described in the results section, raised semi-degraded bog showed the lowest fungal count. Elliott and co-authors demonstrated reduced fungal numbers in degraded peat in their study, which was also congruous with our study (Figure 5a). Apart from the raised semi-degraded bogs, the relative variation of the fungal numbers in cutaway grassland and unimproved grassland was not statistically significant ($p > 0.05$). The identified fungal isolates in our study belonged to phylum Ascomycota, which is in line with a recent study conducted in Australia. The study reported that members of Ascomycota (73%) showed the highest abundance [20].

The anaerobic ecosystem is also a vital part of peatlands. The aerobic habitats might well present opportunities for the growth of the native yet culturable microorganisms present in cutaway and grassland peat (Figure 3a). A previous study on degraded ombrotrophic peatland located in the Southern Pennines, UK found lower bacterial numbers in degraded bare peat compared to any of the vegetated peatlands tested [29]. However, raised semi-degraded bog was consistent with the lowest number of anaerobic bacterial population. Preliminary attempts were taken in this study to explore the anaerobic bacterial population residing in these sites. According to the literature, the acetogenic bacterial group was hypothesized to be the most frequent anaerobes found in peatlands. *Clostridium* sp. is categorized under acetogens and converts CO_2 into acetic acid, which is a key role in the anaerobic environment [33]. Methanogens also have a predominant community present in peat soils. The anaerobic process, which is governed by methanogenic archaea, is not extensively studied. A scientific investigation was conducted to find how methanogenic abundance varies during long-term drainage and rewetting. The study estimated the time taken to retrieve the anaerobic microbial communities after hydrological rewetting [34]. However, our interest in the aspects of anaerobic bacteria was to find information on whether the populations differ with the peat soil type.

The microhabitat associated with some of the higher plants had proven to influence the anaerobic bacterial population in a study carried out in the Czech Republic [35]. If the root system of the plant inhabitants can maintain good aeration, along with rhizodeposition, aerobic growth will be enhanced. Conversely, *Sphagnum*-dominated peat, such as in raised bogs, can create anoxic conditions due to the high water table [35]. *Sphagnum* mosses release substantial amounts of hydrogen, which facilitates the growth of methanogenic organisms [36]. According to Figure 6a, a *Sphagnum*-rich site i.e., raised semi-degraded bog, showed an average number of 2.16×10^4 CFU g^{-1} anaerobic bacteria, which was lower than all the other sites mentioned. This contradicts previous findings [36], in which *Sphagnum* creates a favorable microhabitat for anaerobic growth. This could be explained by most of the anaerobes present in our raised bog not being able to culture in

artificial media. Also, the anaerobic medium is more neutral than the raised bog. It might inhibit the growth of some anaerobes due to the difficulty of adapting to neutral culture conditions. It may be possible to detect the unculturable population using diluted media and long incubation periods. Setting up bioreactors to mimic the actual peat microenvironment is another promising method to isolate unculturable organisms. Our future aspects include developing bioreactors to isolate the unculturable microbial population and combine metagenomic studies to obtain a better picture of peat microbiota.

Another key focus of our study was the isolation of phosphate-solubilizing bacteria (Figure 6b). Continuous addition of phosphate (P) fertilizers into the soil has proven to cause detrimental effects on the environment. Employing PSB as a tool to increase P availability in the soil would be a convenient alternative for commercial phosphate fertilizers. Many studies have presented results in the scientific literature solely around the topic of PSB isolated from tropical peatlands. A very recent study based on PSB revealed the potential of PSB to use as a soil fertilizer. *Staphylococcus* sp., isolated from tropical peat, demonstrated the ability to solubilize $AlPO_4$ at a pH of 5.54. The concentration range of $AlPO_4$ tested was 36.88 to 39.65 ppm [37]. Our study's key substrate was β-tri-calcium phosphate. It was maintained in a constant substrate concentration (5005.71 ppm ≈ 5.0 g/L) at pH 7. Moreover, our study was able to determine the solubility indexes (SI) of PSB isolates across four 'land-use' types (Table 2). PSB from saprists peat soil was isolated, and the SI of these isolates ranged from 2.48 to 5.23 [38]. This was slightly higher than the values reported in our study. This suggests that our study sites with high PSB activity (cutaway and unimproved grassland) might have the potency to act as a biofertilizer. Before the PSB isolates are recommended as a biofertilizer, pot culture experiments and field trials should be carried out. A recent study used peat soil for pot culture studies as a medium for PSB due its chemical properties [39]. However, the same study revealed the SI of phosphate-solubilizing fungal isolates was significantly lower than the bacteria. Whilst there could be a few bacterial mechanisms for phosphate solubilization, secretion of organic acids is considered the prime mechanism [40].

The actinomycetes group is vital for the decomposition process in the peatland ecosystem. In all the study sites, actinomycetes were morphologically and microscopically detected. The colonies grew after a 14-day incubation period. They showed a characteristic halo zone around the colonies. This could be due to the hydrolysis of the starch component by the actinomycetes. This was confirmed after the addition of iodine into Petri dishes. The SCA plate turned a blue color, while the halozone remained clear (Figure 7a). Another basic morphological characteristic was the presence of substrate mycelium and aerial mycelium [41], which were detected in isolates from our study sites (Figure 7b). The emergence of colorful colonies could be due to the production of pigments (Figure 7d,e). However, our study did not quantify the colonies on SCA as an overall actinomycete number because some colonies might represent saccharolytic organisms. Therefore, presuming all the colonies were produced by actinomycetes would be inaccurate. Although actinomycetes were not broadly studied earlier, the recent developments in next-generation sequencing [42] have paved the way for many approaches to investigate the genome of novel actinomycete species. One such instance was reported in Thailand, where a novel actinomycete strain was isolated from a peat swamp. It belongs to the family Actinomycete. The study was based on this organism and detailed morphological, cultural, physiological and molecular analyses were conducted [42]. Similarly, novel *Streptomyces* spp. was extracted from peat swamp forest soil. Starting from the 16S rRNA gene analysis, cultural biochemical characters and chemotaxonomy have been analyzed [43]. In another study, which was carried out in China [44], the total concentration of the actinomycetes was lower than bacteria, according to PLFA results. Other work has demonstrated the role of actinomycetes in decomposition [45] when overall bacterial growth starts to decrease. This is because as the decomposition takes place, the nutrients that are available for the bacteria also decrease. Bacteria are mainly able to act as soil decomposers in peatlands at the initial stages [46]. Actinomycetes carry out decomposition in the latter stage.

According to the results discussed in Section 3, the aims and objectives of this pilot study were achieved. Preliminary data were gathered to continue studies and initiate future rewetting schemes. The microbial parameters mentioned here have the possibility of being employed as early indicators to monitor restoration progress. Unculturable microbial populations in these lands can be studied in the future by setting up bioreactors to mimic the peat environments. Microbial respiration would be a promising future direction as it is an indication of microbial activity. Microbial activity can be compared in peatlands before and after the restoration process. Since drained peatlands contribute to greenhouse gas emissions, nitrification genes of microbes can be quantified as it implies the availability of oxygen in peatland. In addition to bacteria and fungi, prokaryotic community structure in peatland can be studied as it is more responsive to the environmental changes caused by 'land use'. This pilot study does not anticipate being a conclusive study of peatland microbiology, but the findings will pave the way for future directions in peatland microbiology.

5. Conclusions

According to this study, the tested Irish peatlands with different 'land-use' types contained distinct numbers of culturable viable microorganisms (aerobes, fungi, anaerobes, actinomycetes and phosphate-solubilizing organisms). There was a statistically significant difference between the microbial numbers in unimproved grassland and raised semi-degraded bog. pH in peat samples could be one reason to obtain significantly different microbial numbers in cutaway and raised semi-degraded bog compared to the rest. Cutaway bog contained the bacteria with the highest phosphate-solubilizing activity. However, actual microbial populations could be higher than the values presented in this study. *Penicillium* spp. and *Trichoderma* spp. were common in all the sites. Overall, the microbial numbers in cutaway and raised semi-degraded bog statistically varied, while the rest of the sites did not change drastically ($p \leq 0.05$) in their microbial numbers according to 'peatland-use' type. The fulfilled aims and objectives of this pilot study are also beneficial to set up promising rewetting schemes to reduce greenhouse gas emissions because the outcome of the rewetting and reclamation depends on how those practices enhance the interaction between microbial communities and environmental variables, such as temperature, pore water pH and plant species richness. Aboveground vegetation greatly shapes microbial function. Microbial community structure in combination with soil chemistry and biogeochemical process can provide better conclusions on how effective restoration practices can be. Agronomical and ecological advantages can be expected if this study continues. Furthermore, study gaps regarding soil and plant heath can be minimized in terms of organic agriculture.

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