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Effects of the Freezing–Thawing Cycle Mode on Alpine Vegetation in the Nagqu River Basin of the Qinghai–Tibet Plateau

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Abstract: The freezing–thawing cycle is a basic feature of a frozen soil ecosystem, and it affects the growth of alpine vegetation both directly and indirectly. As the climate changes, the freezing–thawing mode, along with its impact on frozen soil ecosystems, also changes. In this research, the freezing–thawing cycle of the Nagqu River Basin in the Qinghai–Tibet Plateau was studied. Vegetation growth characteristics and microbial abundance were analyzed under different freezing–thawing modes. The direct and indirect effects of the freezing–thawing cycle mode on alpine vegetation in the Nagqu River Basin are presented, and the changing trends and hazards of the freezing–thawing cycle mode due to climate change are discussed. The results highlight two major findings. First, the freezing–thawing cycle in the Nagqu River Basin has a high-frequency mode (HFM) and a low-frequency mode (LFM). With the influence of climate change, the LFM is gradually shifting to the HFM. Second, the alpine vegetation biomass in the HFM is lower than that in the LFM. Frequent freezing–thawing cycles reduce root cell activity and can even lead to root cell death. On the other hand, frequent freezing–thawing cycles increase microbial (*Bradyrhizobium*, *Mesorhizobium*, and *Pseudomonas*) death, weaken symbiotic nitrogen fixation and the disease resistance of vegetation, accelerate soil nutrient loss, reduce the soil water holding capacity and soil moisture, and hinder root growth. This study provides a complete response mechanism of alpine vegetation to the freezing–thawing cycle frequency while providing a theoretical basis for studying the change direction and impact on the frozen soil ecosystem due to climate change.

Keywords: freezing–thawing cycle mode; aboveground and underground biomass; soil microbe; soil chemical properties; climate change

1. Introduction

Vegetation is an important part of the soil ecosystem and is critical for soil and water conservation, soil remediation, the development of animal husbandry, and ecosystem function and services [1–6]. As a special soil ecosystem [7,8], the frozen soil ecosystem contains unique alpine vegetation [9,10]. Alpine vegetation is sensitive to environmental factors [11–14], and the freezing–thawing cycle is the most important environmental factor affecting vegetation growth and survival in alpine regions.

Consequently, alpine vegetation is a useful indicator of changes in environmental factors due to climate change.

The freezing–thawing cycle (FTC) is a phenomenon in which the soil undergoes repeated freezing and melting as a result of seasonal or diurnal temperature change [15]. Frequent low-temperature disturbances can damage vegetation roots and directly affect vegetation growth [16–18]. Among the indirect effects of FTC are its impacts on the physical and chemical properties of soil [19–26], microbial communities (including their composition) [27–30], greenhouse gas emissions [31,32], the soil structure, and the distribution of water and nutrients. As the climate warms, the freezing–thawing cycle mode (FTCM) changes [33–36] (FTCM is a general term for all characteristics of the FTC), thus affecting vegetation growth characteristics and growth trends; the effect even extends to the entire frozen soil ecosystem. Due to the sensitivity and vulnerability of frozen soil ecosystems [37–40], the effects of climate change are magnified and the process of ecosystem change may be shortened, which has adverse effects.

In this research, we visualized the influence of FTCM on the underground parts of vegetation. FTCM primarily affects the soil vegetation, either directly or indirectly, by changing the vegetation roots, the physical and chemical properties of the soil, and the soil microorganisms. As shown in Figure 1, five influencing pathways are proposed (the different colors represent different pathways of influence). Some of the mechanisms have been confirmed, while others have not been studied or are not well understood.

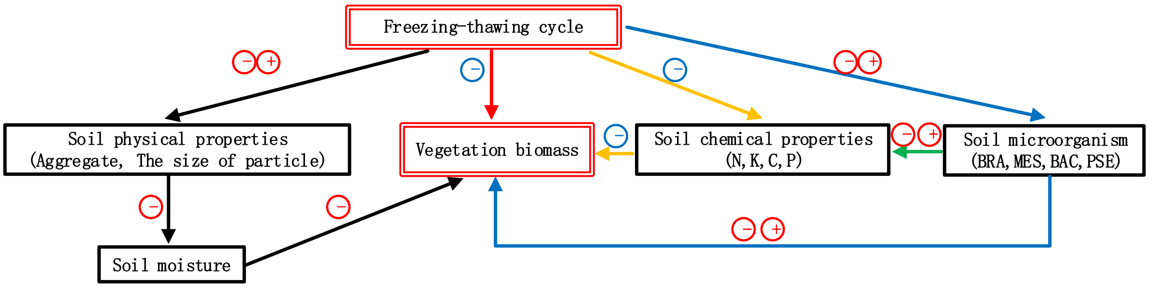

Figure 1. The theories and assumptions of the direct and indirect effects of the freezing–thawing cycle (FTC) on alpine vegetation. The lines with different colors indicate different ways that the freezing and thawing cycle affect vegetation. The '+' sign indicates a positive correlation, the '−' sign indicates a negative correlation, the blue symbol indicates that the corresponding theory has been confirmed, and the red symbol indicates that the corresponding theory is unconfirmed.

FTC is often accompanied by low temperatures and repeated low-temperature disturbances. The low-temperature environment reduces the activity of vegetation root cells and slows the rate of nutrient uptake by roots [16,41]. At the same time, roots adopt anaerobic respiration, accumulate anaerobic respiration products, and reduce their physiological activity [4]. Lower temperatures can even lead to phase changes in vegetation root cell membranes, destroying the cell structure [42]. Moreover, the requirements of vegetation root adaptation to frequent and severe low-temperature disturbances are high [43], which further leads to vegetation growth being inhibited or even destroyed. As shown by the red line in Figure 1, this phenomenon has been confirmed.

The FTC also has indirect impacts on vegetation growth and survival. Most microorganisms have reduced cell activity in a low-temperature environment [44], and their cell structures can even be destroyed [27]. Frequent FTCs can directly damage the cell membrane and cell wall of microorganisms [30,45], leading to microbial death and the release of carbon, nitrogen, and other nutrients [46]. As a result, frequent FTCs may affect the survival of these plant growth-promoting rhizobacteria, which can affect their capabilities to decompose matter and promote nitrogen fixation and disease resistance [47–52]. In turn, the nutrient content in the soil is affected, and ultimately, the growth of vegetation in the soil is influenced, as shown by the blue and green lines in Figure 1. Meanwhile, the increased frequency of FTCs accelerates the loss of nutrients in the soil [19,20], possibly leading to a reduction in the total nutrient content in the soil, which is not conducive to alpine vegetation growth. The yellow line in Figure 1 represents this effect, which has also been confirmed. Furthermore, the volume of the soil water expands when it freezes and decreases when it thaws. These changes decrease the size of the soil aggregates [22,23] and change the soil porosity and bulk density [53]. These effects change the water holding capacity and water conductivity of the soil. The soil moisture changes and affects vegetation growth, which is represented by the black line in Figure 1.

Current studies have focused on the relationship between vegetation and low temperatures or FTCs. However, studies on the response of the underground part of vegetation to the frequency of freezing and thawing cycles are lacking, and studies on the effects of changes in FTCM on vegetation due to global climate change are even rarer [54]. Thus, we present five hypotheses: (i) the FTCM directly affects alpine vegetation roots; (ii) the FTCM affects plant growth-promoting rhizobacteria, and subsequently alpine vegetation; (iii) the FTCM affects the physical properties of soil, which in turn affect the soil water content and ultimately alpine vegetation; (iv) the FTCM affects soil microbes and soil physical properties, which in turn affect the soil chemical properties and ultimately alpine vegetation; and (v) the effect of the FTCM on alpine vegetation is a complete, indispensable system; moreover, as the FTCM changes under climate change, the alpine vegetation must also be affected. Our approach was to combine physics, chemistry, biology, and microbiology methods to help better understand the mechanism of the direct and indirect effects of the FTCM on alpine vegetation.

2. Materials and Methods

2.1. Study Area

The Nagqu River Basin (Figure 2) is located in the northern part of the Tibet Autonomous Region and is surrounded by the Tanggula Mountains, the Nyainqentanglha Mountains, and the Gangdisi Mountains. The Nagqu River Basin is the source of the Nujiang River, which is a transition region for the Indian Ocean monsoon and westerlies.

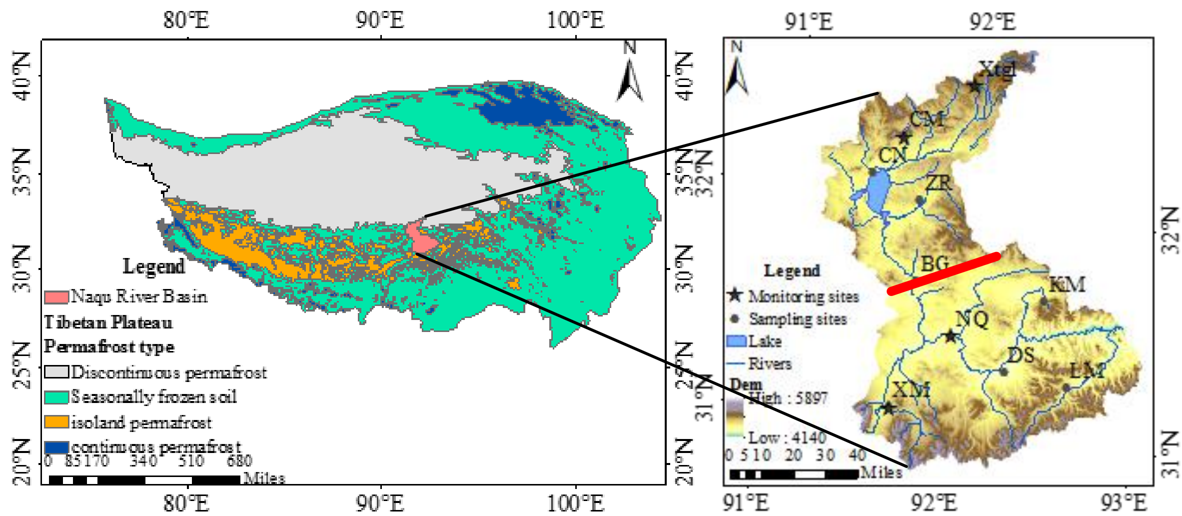

Figure 2. The location map of the Nagqu Basin. The star-shaped points are the monitoring sites, and the dots are the sampling sites. The different colors in the (**left**) figure represent different types of frozen soil, and the different colors in the (**right**) figure represent different elevations.

The majority of the Nagqu River Basin is made up of felty soils, with an area of 11,018.3 km^2, accounting for 67.5% of the basin area. The soil in the basin is mainly frigid frozen soils above the snow line and the ice margin, the surrounding water system is mainly made up of meadow soils. Other areas are also distributed with bog soils, alluvial soils, and other soils (Table 1). The main vegetation in the

Nagqu River Basin includes *Kobresia bellardii*, *Kobresia humilis*, and *Kobresia parva*. Alpine matted dwarf shrubs dwell in the mountainous areas, while some alpine sparse plants such as *Saussurea medusa* Maxim and *Saussurea japonica* are found in the high mountainous areas [55].

Table 1. Site information and soil property information.

Sites	Altitude (m)	Vegetation Coverage (%)	Soil Bulk Density (g/cm^3)	Soil Porosity (%)
Xtgl	5050	10	1.34	67.93
CM	4760	70	1.26	79.13
CN	4580	90	1.30	72.63
ZR	4660	60	1.32	62.20
BG	4560	20	1.51	55.53
NQ	4560	35	1.29	51.67
XM	4730	60	1.29	71.77
DS	4550	80	1.38	46.40
LM	4640	90	1.49	63.60
KM	4710	80	1.37	43.50

2.2. Data Collecting and Processing

2.2.1. Soil Temperature and Volumetric Water Content Data

With the support of the National Natural Science Foundation of China, four monitoring sites have been set up in Cuoma Township (CM), Xiangmao Township (XM), Xiaotanggula Mountain (Xtgl), and Nagqu Bridge (NQ) since October 2016. The 5-cm soil temperature and volumetric water content data (recorded every 30 min) from 1 January to 30 June 2018 were obtained from four monitoring sites; the 5-cm soil temperature and moisture data (recorded every 30 min) from 1 January to 30 June in 2011 and 2014 were obtained from studies by Yang Kun et al. [56–59].

Our instrument for measuring the soil temperature and volumetric water content (VWC) was the SM300 soil moisture sensor from Delta, UK, which has an accuracy of ±0.1 °C and ±0.1% VWC. The sensor was buried in the soil to a depth of 5 cm. The instruments used by Yang Kun et al. were the EC-TM and 5TM from the USA; this sensor was buried in the soil to a depth of 5 cm and used to simultaneously measure the soil temperature and VWC with an accuracy of ±1 °C and ±2% VWC.

2.2.2. Vegetation Biomass Data

In August 2018, the aboveground and underground biomass were measured in Xiangmao Township (XM), Dasa Township (DS), Luomai Township (LM), Nagqu Bridge (NQ), Bange Bridge (BG), Kongma Township (KM), Zharen Town (ZR), Cuona Lake (CN), Cuoma Township (CM), and Xiaotangula Mountain (Xtgl) through a direct acquisition method. The root system of the Nagqu River Basin is generally concentrated around 10 cm [60]. Three sample plots of 20 × 20 cm were selected for each sampling site, and clods with a height of 20 cm were excavated. The leaves of the vegetation (i.e., aboveground parts) were cut off, and the roots of the vegetation (i.e., underground parts) were separated from the clods. These were placed in sealed bags and oven-dried at a constant temperature of 65 °C for 48 h. The dried samples were then weighed on an electronic balance. The dry weight of the aboveground and underground parts of the vegetation is the aboveground and underground biomass [61].

2.2.3. Soil Chemical Properties

Soil samples from the 0–10 cm soil layer were collected in XM, DS, LM, KM, ZR, CN, CM, and Xtgl in May 2018. The soil samples were measured by the Beijing Academy of Agriculture and Forestry Sciences according to GB/T (National standard in China), HG/T (Chemical Industry Standards in China), and other national standards, as well as ASTM (American Society of Testing), ISO (International Organization for Standardization), EN (European Norm), and other international standards. Soil moisture, available phosphorus (AP), hydrolyzable nitrogen (HN), available K (AK), microbial biomass carbon (MBC), and other information were also obtained.

2.2.4. Soil Microbial Data

In May 2018, microbial information from the 0–10 cm soil samples from NQ, XM, CM, and Xtgl was collected by the Shanghai Meiji Biological Company. Meiji Biological Company used a third-generation sequencing platform to sequence the 16S rDNA/18S rDNA/ITS/functional genes and other specific segments using their PCR (Polymerase chain reaction) products. Information on the microbial community structure, evolutionary relationship, and the correlation between the microorganisms in the soil samples and environment were obtained.

2.3. Freezing–Thawing Cycle Index Calculation

The FTC is a key feature of seasonally frozen soil ecosystems. It affects not only the physical and chemical properties of the soil [19–26], but also the survival of the vegetation and microorganisms in the soil [16,17,27–29]. Measuring the impact of FTCs on vegetation and soil is challenging. The freezing–thawing cycle days (FTCD) and the number of freezing–thawing cycles (NFTC) [62–65] can reflect the freezing–thawing characteristics of the soil. However, if only the FTCD value is considered, the influence of FTC characteristics may be neglected; if only the NFTC value is considered, the intensity of the FTCs and the influence of the freezing–thawing duration may be neglected. Therefore, in this study, the FTCD and NFTC were combined. The freezing–thawing cycle frequency (FTCF) index was defined to measure the effect of FTCs on alpine vegetation and can be expressed by

$$\mathrm{FTCF} = \frac{\mathrm{NFTC}}{\mathrm{FTCD}} \quad (1)$$

3. Results

3.1. Freezing–Thawing Cycle Characteristics

FTCs mainly occur during the thaw initiation period and the freeze initiation period [66,67]. The FTC data that were used to determine the impact on plant growth were from 1 January to 30 June (in the following, unless otherwise specified, the FTCs occurred in the first half of the year). The soil temperature and moisture data from XM, NQ, CM, and Xtgl from 1 January to 30 June 2018 were analyzed (Table 2). The starting and closing times of the topsoil FTCs at the four monitoring sites differed within a large range. XM had the largest NFTC and FTCD values. Compared with XM, the FTCD values in NQ, CM, and Xtgl were 67.9%, 64.1%, and 66.7% lower, respectively. The NFTC values in NQ, CM, and Xtgl were 67.5%, 85%, and 75% lower, respectively. The FTCF index values in XM and NQ were larger than one, while those in CM and Xtgl were less than one (Table 2).

Table 2. Freezing-thawing cycle characteristics of topsoil at four monitoring sites from January to June 2018.

Monitoring Sites	Starting Time	Closing Time	NFTC	FTCD	FTCF
XM	9 January 2018	28 March 2018	80	78	1.02
NQ	24 February 2018	20 March 2018	26	25	1.04
CM	8 February 2018	8 March 2018	12	28	0.43
Xtgl	28 March 2018	22 April 2018	20	26	0.77

The FTC of the topsoil has an important relationship with altitude and vegetation coverage [68,69]. Altitude controls the air temperature, which in turn affects the FTCF, and vegetation coverage affects the range and rate of the soil temperature change, which in turn affects FTCF. In the Nagqu River Basin, BG had the lowest altitude of 4560 m, and the vegetation coverage there was very low, at only 20%. There was a significant regularity in the distribution of altitude and vegetation coverage. (Table 1). Therefore, BG is a key point. In this study, BG was used as a boundary, and the freezing–thawing frequency was considered equal to one as the standard. The FTC of the Nagqu River Basin was divided into a high-frequency mode and a low-frequency mode (in the right panel in Figure 1, the high-frequency mode is below the red line and the low-frequency mode is above the red line).

The FTCF is used as an important indicator to measure the effects of FTCs on alpine vegetation. There are significant spatial differences in the Nagqu Basin. With global warming, the frozen soil ecosystem of the Qinghai–Tibet Plateau has different degrees of degradation [70,71]. The FTCF is also changing, so the temporal change in the FTCF also needs to be analyzed.

The characteristics of the topsoil FTCs in XM and NQ in 2011, 2014, and 2018 were analyzed (Table 3). Table 3 shows that the starting and closing times of the FTCs were earlier, and the overall FTC had a significant upward trend. This phenomenon suggests that climate warming has led to an overall increase in soil temperature, which has changed the start and duration of each period in the freezing–thawing process. These changes will have adverse effects on the frozen soil ecosystem that will lead to its destruction. The FTCF is also an indicator that is used to measure the degradation of the frozen soil ecosystem and how it is affected by the freezing–thawing process and climate change. The FTCM tended to transition from the low-frequency mode (LFM) to the high-frequency mode (HFM).

Table 3. The characteristics of the topsoil freezing-thawing cycles from January to June in Xiangmao Township (XM) and Nagqu Bridge (NQ) in 2011, 2014, and 2018.

Monitoring Sites	Starting Time	Closing Time	NFTC	FTCD	FTCF
XM	16 March 2011	17 April 2011	15	32	0.47
	16 March 2014	4 April 2014	10	20	0.50
	9 January 2018	28 March 2018	80	78	1.02
NQ	1 April 2011	17 April 2011	8	17	0.47
	27 February 2014	25 March 2014	19	27	0.70
	24 February 2018	20 March 2018	26	25	1.04

3.2. Microbial Characteristics

Soil microbes play an important role in the conversion of material and energy in soil ecosystems [72,73], and the unique FTC characteristics of the Qinghai–Tibet Plateau provide a complex environment for microbial growth [74]. In this study, soil microbes were detected in XM, NQ, CM, and Xtgl. The results are shown in Figures 3 and 4.

The main soil microbes in the Nagqu River Basin were Actinobacteria, Proteobacteria, Acidobacteria, and Chloroflexi. Among them, Actinobacteria and Proteobacteria were the dominant flora in the whole basin. Acidobacteria and Chloroflexi were also dominant in some areas, but the relative abundance of Acidobacteria and Chloroflexi in CM was relatively low. Yang [74] and others found that Actinobacteria and Proteobacteria were the dominant flora in the Tuotuo River Basin of the Qinghai–Tibet Plateau. Zhang et al. [75] studied the FTCs of the Beilu River in the Qinghai–Tibet Plateau and reported that Proteobacteria and Acidobacteria were the dominant flora in the study area. Moreover, Proteobacteria and Acidobacteria were found to be the dominant flora in the Colorado Rocky Mountains and the Arctic permafrost regions [76,77]. Jansson et al. also summarized the main categories of microbes in frozen soil [78]. The findings from those studies are consistent with the results of this study, confirming that Actinobacteria, Proteobacteria, and Acidobacteria are typical residents in a cold soil environment and have good adaptability to an environment that is characteristic of alpine regions.

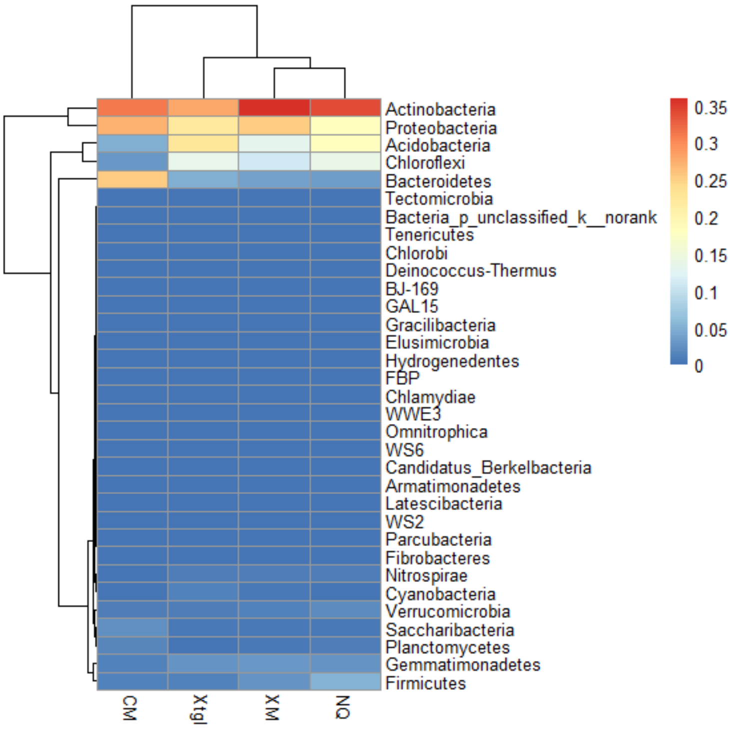

Figure 3. The relative abundance of soil microbes in XM, NQ, Cuoma Township (CM), and Xiaotanggula Mountain (Xtgl). The different colors indicate different relative abundances. The rows specify bacteria and the columns indicate the sampling sites.

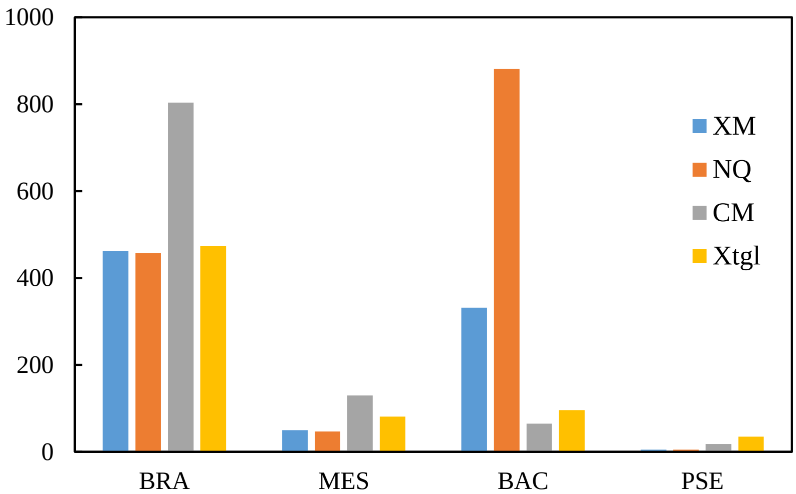

Figure 4. Distribution of plant growth-promoting rhizobacteria at different sites, *BRA* is *Bradyrhizobium*, *MES* is *Mesorhizobium*, *BAC* is *Bacillus*, and *PSE* is *Pseudomonas*.

The soil in the Nagqu River Basin also contained plant growth-promoting rhizobacteria such as *Bradyrhizobium* (*BRA*), *Mesorhizobium* (*MES*), *Bacillus* (*BAC*), and *Pseudomonas* (*PSE*) species. Among them, *BRA* and *MES* can be symbiotic with plant roots and promote nitrogen fixation and the absorption of nitrogen by vegetation [47,48]. *BAC* has a strong ability to decompose organic matter, thus improving soil fertility and promoting natural material circulation [49–51]. *PSE* can control vegetation diseases and promote vegetation growth [52]. Soil and microbial data at XM, NQ, CM, and Xtgl were analyzed (Table 4 and Figure 4), where the top soil temperatures (minimum, maximum, average) at the two sites of CM and XM were similar, but the microbial distribution was very different. From Table 4 and

Figure 4, there was no significant relationship between the soil temperature and microbial community at four sites, but the FTCF and the distribution of microbial community were very close. This indicates that microbes in alpine regions have adapted to the extreme temperature of the soil, but the FTCF may have a greater impact on the survival of microorganisms, which is consistent with the views of Yang et al. [30].

Table 4. Soil temperature information and the freezing–thawing cycle frequency at each site.

Site	Minimum Soil Temperature	Maximum Soil Temperature	Average Soil Temperature	FTCF
XM	−6.6	16.2	5.4	1.02
NQ	−9.9	10.7	−1.1	1.04
CM	−6.6	16.4	5.5	0.43
Xtgl	−15.9	16.0	0.4	0.77

Figure 5 shows that the abundance of *BRA*, *MES*, and *PSE* was lower in the HFM than in the LFM, but *BAC* was higher in the HFM than in the LFM. These results indicate that although *BRA*, *MES*, and *PSE* have strong cold resistance, they may be damaged by frequent low-temperature disturbances. Meanwhile, *BAC* has good environmental adaptability. Moreover, the HFM may destroy the cellular structure of bacteria such as *BRA*, *MES*, and *PSE*, and their destruction releases their nutrients and reduces the competition for nutrients in the soil. There are more available nutrients that can be absorbed by *BAC*, so the abundance of *BAC* is higher in the HFM.

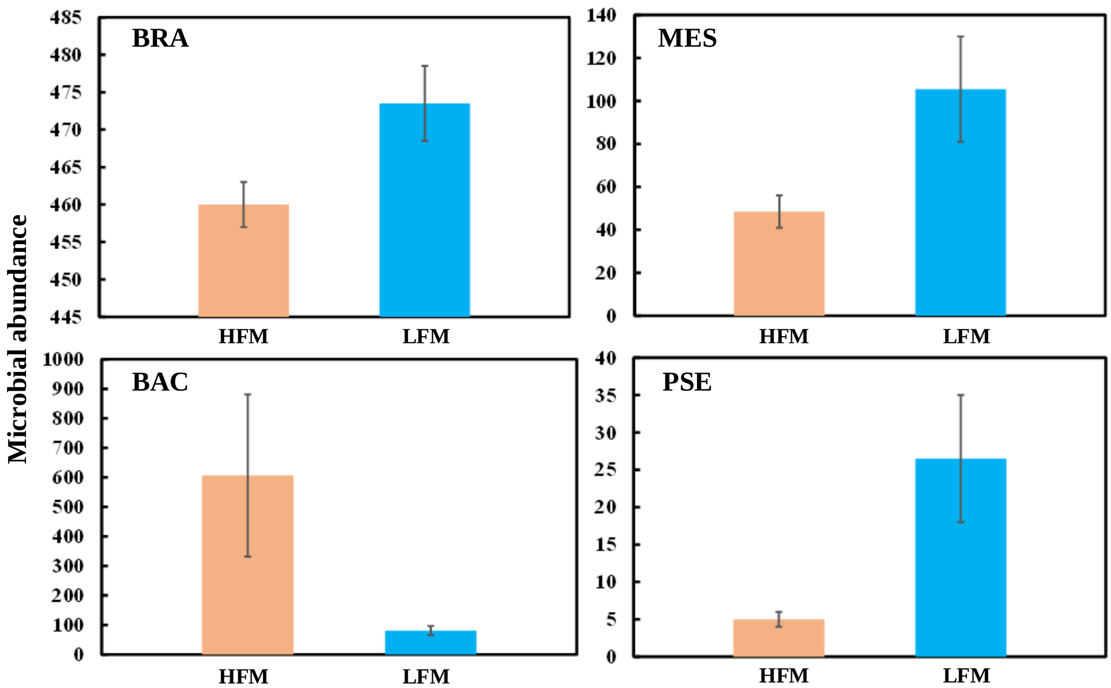

Figure 5. The average abundances of *Bradyrhizobium* (*BRA*), *Mesorhizobium* (*MES*), *Bacillus* (*BAC*), and *Pseudomonas* (*PSE*) species in different freezing–thawing cycle modes (FTCMs). The vertical line indicates the error limit, HFM is the high-frequency mode, and LFM is the low-frequency mode.

3.3. Aboveground and Underground Biomass

In this study, the biomass of three plots at each sampling site was averaged, and the aboveground and underground biomass in different FTCMs were analyzed (Figure 6). In the Nagqu River Basin, the aboveground and underground biomass of the vegetation in the HFM were reduced when compared with those in the LFM. Frequent low-temperature disturbances destroy vegetation root cells [43], may kill some species of microorganisms that are beneficial to vegetation growth, and inhibit the growth of vegetation.

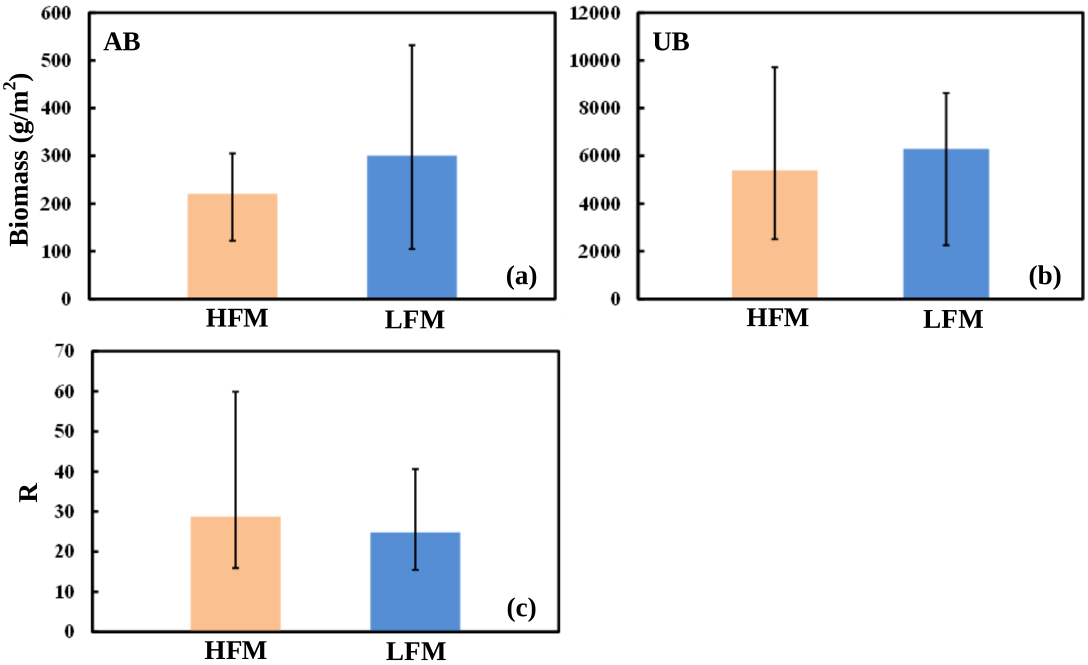

Figure 6. The aboveground (**a**) and underground (**b**) biomass of the Nagqu River Basin; (**c**) the value of R in the Nagqu River Basin. The vertical line indicates the error limit, HFM is the high-frequency mode, and LFM is the low-frequency mode.

Although vegetation growth is affected by a series of factors including the FTC, there was a good correlation between the aboveground and underground biomass. Moreover, these factors followed the same increasing or decreasing trend in different environments. The R of vegetation in the Nagqu River Basin was much larger than 1.

The vegetation in this area uses most of the net production to develop root systems, indicating that the vegetation is highly competitive for water and nutrients, and extensive root systems are conducive to their survival in a relatively poor and harsh environment. At the same time, the proportion of the aboveground part is small, which can reduce the water lost by evaporation, which is consistent with the growth characteristics of vegetation in most parts of the Qinghai–Tibet Plateau. However, there was no significant change in R in different FTCMs (Figure 5), indicating that R is affected by the vegetation species and growth characteristics, while the FTCM has little or no effect on this parameter.

3.4. Correlation Analysis

3.4.1. Short-Term Fluctuation Analysis

The underground biomass data that were obtained from monitoring or experimentation in 2018 included soil available phosphorus, hydrolyzable nitrogen, available K, microbial biomass carbon, the number of freezing–thawing cycles, the freezing–thawing cycle days, the freezing–thawing cycle frequency, and the daily average temperature difference as well as the abundance of *Bradyrhizobium*, *Mesorhizobium*, *Bacillus*, and *Pseudomonas* species. The correlation analysis graph in shown in Figure 7.

In Figure 7, it is shown that the vegetation biomass decreased with the increase in the FTCD value, but the inverse relationship between the vegetation biomass and the FTCF was more significant. The index of the FTCF was assumed to be highly advantageous for evaluating the influence of FTCs on vegetation, and the results were consistent with this assumption. Moreover, the nitrogen fixation by *BRA* and *MES*, the decomposition by *BAC*, and the growth promotion by *PSE* had a non-negligible effect on vegetation, and their responses to the FTCM were different (see the analysis in Section 3.3). FTCs destroy the soil structure, resulting in a decrease in the soil water content, which in turn affects vegetation growth. This result was confirmed by a long-term fluctuation analysis, which is presented in Section 3.4.2.

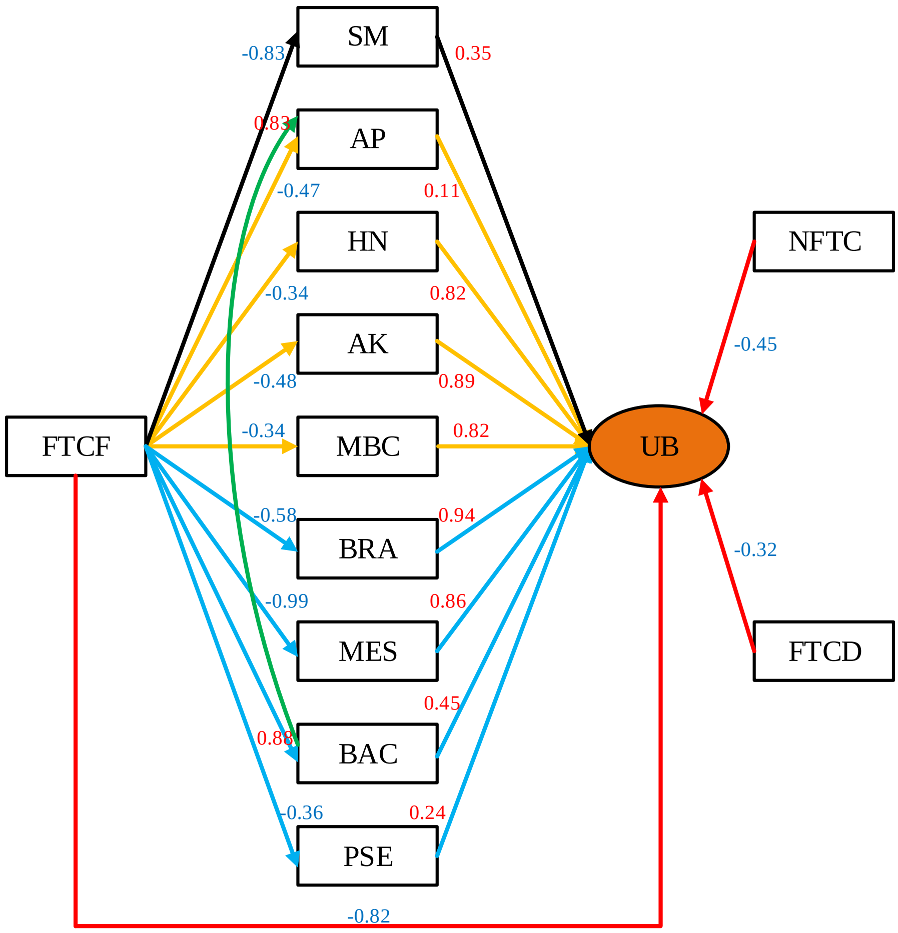

Figure 7. Short-term correlation analysis, UB is underground biomass, AP is available phosphorus, HN is hydrolyzable nitrogen, AK is available K, MBC is microbial biomass carbon, NFTC is the number of freezing–thawing cycles, FTCD is the number of freezing–thawing cycle days, FTCF is the freezing-thawing cycle frequency, *BRA* is *Bradyrhizobium*, *MES* is *Mesorhizobium*, *BAC* is *Bacillus*, *PSE* is *Pseudomonas*, and SM is soil moisture. The colors of the arrows correspond to the colors of the lines in Figure 1. The same color indicates the same direction, blue words indicate a negative correlation, red words indicate a positive correlation, and the above correlation coefficients were all found to be statistically significant at the $P = 0.05$ level.

The direct impact of an FTC on vegetation and its indirect effect on vegetation through the alteration of nutrient and moisture content in the soil are consistent with the original assumptions of this study. In general, FTCs are detrimental to the growth of vegetation, and the increased frequency of FTCs may amplify their adverse effects, resulting in a decrease in vegetation biomass.

3.4.2. Long-Term Fluctuation Analysis

As shown in Figure 8, the influence of the change in the FTCF on soil moisture was analyzed for XM and NQ using the soil moisture data from 1 January to 30 June in 2011, 2014, and 2018.

As the pre-conditions and experimental methods differ between studies, the changes in soil aggregates, porosity, and bulk density vary with different initial conditions. Many scholars have different opinions on the effects of FTCs on the physical properties of soil [23–25]. Therefore, this study directly analyzed the response of soil moisture to the FTCF and then analyzed its impact on alpine vegetation.

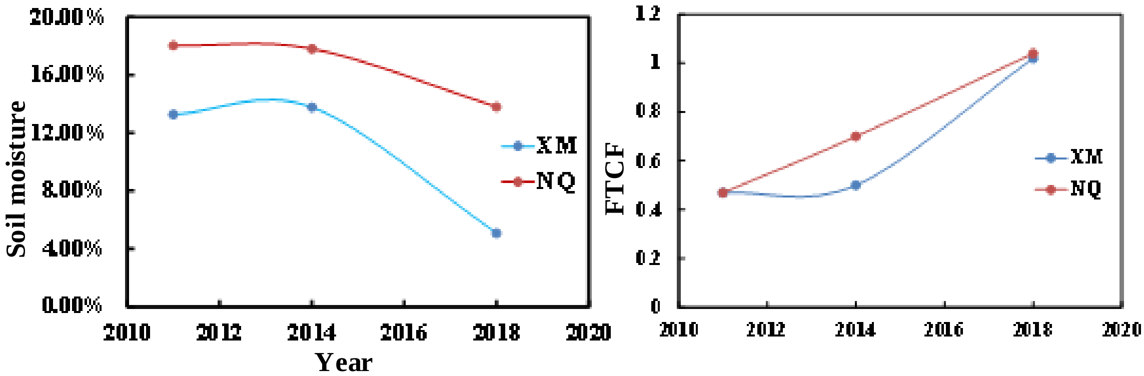

Figure 8. The changes in soil moisture and the FTCF in XM and NQ in 2011, 2014, and 2018.

The FTCM in NQ and XM was the HFM; however, as reported in Section 3.1, these regions experienced a transition from the LFM to the HFM. Figure 7 shows that as the FTCM changed, soil moisture gradually decreased; the correlation among these data was highly significant, which is consistent with the short-term fluctuation analysis and our hypotheses. The comprehensive results show that the greater the FTCF, the greater the damage to soil aggregates, and the higher the rate of soil moisture reduction.

4. Discussion

The mechanism of the direct and indirect effects of the FTCF on alpine vegetation were obtained by drawing from a large number of theories, assumptions, experiments, and analyses (Figures 1, 7 and 9). In the Nagqu River Basin of the Qinghai–Tibet Plateau, the increase in the FTCF amplified the effects of FTCs on vegetation. Moreover, the damage to the vegetation caused by FTCs was continuous (Figure 6). The longer the duration of the FTC, the greater the ratio of metabolically degraded or even damaged cells, which exacerbates the adverse effects on vegetation. Furthermore, the greater the FTCF, the greater the direct damage to the vegetation roots, which may result in decreased vegetation biomass (as shown by the red line in Figure 9).

For soil microbes in the Nagqu River Basin, *BRA*, *MES*, and *PSE* species cells are usually damaged since the increase of the FTCF weakens the symbiotic nitrogen fixation and disease resistance of vegetation, resulting in negative impacts on vegetation growth (as shown by the blue line in Figure 9). However, the death of *BRA*, *MES*, and *PSE* species leads to the release of their nutrients and increases the nutrient content in the soil. The nutrient available for absorption by *BAC* increases, and thus the abundance of *BAC* species increases, which in turn increases soil fertility (as shown by the green line in Figure 9). Although the nutrients in the soil may increase due to the soil microbes, the increase in the nutrient content is not large enough to compensate for the loss and freezing. The total nutrient content in the soil is reduced, so there is an overall detriment to the growth of vegetation (as shown by the yellow line in Figure 9). Moreover, as the FTCF increases, soil aggregates may become more damaged, soil moisture decreases, and vegetation growth is inhibited (as shown by the black line in Figure 9).

According to the IPCC (Intergovernmental Panel on Climate Change) report, the global average temperature will increase by 1.5–3.5 °C in the next 50–100 years [79]. Continued warming of the climate will result in the FTCM transitioning from the LFM to the HFM. The HFM is more damaging to vegetation roots, and this change will inhibit vegetation growth. The reduction or disappearance of the LFM will change the composition of the microbial flora in the Nagqu River Basin. The abundance of *BRA*, *MES*, and *PSE* species will decrease, while that of the *BAC* species will increase, and the proportion of nutrients absorbed by the vegetation roots will increase. At the same time, changes in the FTCM will affect the distribution of nutrients in the soil, and nutrient sources such as organic matter will gradually shift from the top layer to deeper soil layers [80], which may alter the original growth trends of the vegetation. Moreover, large soil aggregates will break into smaller agglomerates, and fine particles will become medium-sized particles [26]. As the FTCF increases and the duration of the FTCs becomes longer, all the particles in the soil will become medium in size. These effects will change the original growth environment of the vegetation, making the survival of alpine vegetation more difficult.

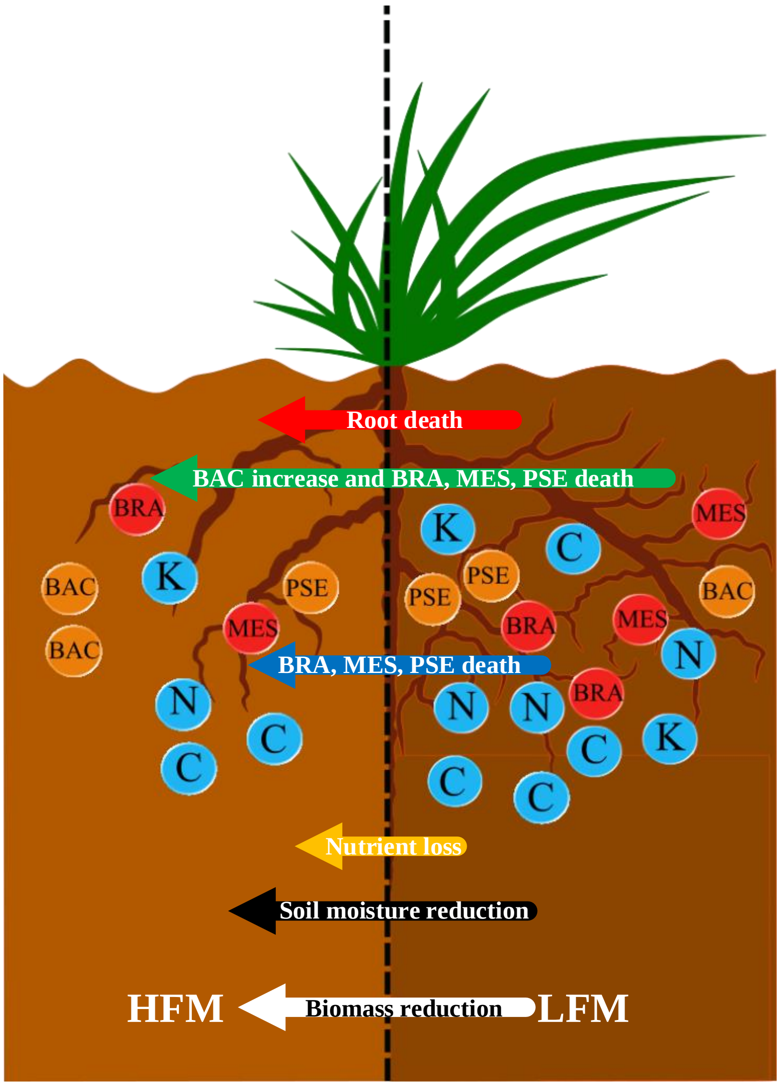

Figure 9. The direct and indirect effects of the FTCM on alpine vegetation. The white arrows indicate the total impact of the transition from the LFM to the HFM on soil vegetation; the arrows of different colors (except white) indicate the different ways in which the FTCM affects alpine vegetation. The colors of the arrows correspond to the colors of the lines in Figure 1. The same color indicates the same direction. The lines in Figure 1 are conceptual, while the arrows in Figure 8 are conclusions based on results.

If all these changes are irreversible, the mechanism driving the material and energy cycle of the ecosystem will change, the climate environment may worsen, living organisms will be subjected to natural selection, and species that cannot adapt to the change will be eliminated. The original ecosystem of the Nagqu River Basin may be completely devastated. Due to the vulnerability of frozen soil ecosystems [37–40], all effects may be amplified, the process of change may be shortened, and a series of unforeseen events may occur.

5. Conclusions

Through the analysis of freezing–thawing cycle characteristics, this study showed that the freezing–thawing cycle mode fluctuates between the HFM and the LFM in the Nagqu River Basin of the Qinghai–Tibet Plateau. The distribution characteristics of the aboveground and underground biomass and soil microbial abundance under different freezing–thawing cycle modes were analyzed, and the direct and indirect effects of the freezing–thawing cycle mode on soil vegetation were revealed. The changes and hazards of freezing–thawing cycle modes due to climate change were discussed, and the following conclusions were drawn:

(1) The freezing–thawing cycle modes in the Nagqu River Basin are the HFM and the LFM. With the influence of climate change, the LFM is gradually shifting to the HFM.
(2) The alpine vegetation biomass in the HFM is lower than that in the LFM. Frequent freezing–thawing cycles may lead to a reduction in root cell activity and even the death of root cells; on the other hand, high-frequency freezing–thawing cycles usually cause the death of certain microorganisms (*BRA*, *MES*, *PSE*) and weaken the roots' nitrogen fixation ability and disease resistance. Moreover, the loss of soil nutrients is accelerated, the soil water holding capacity and soil moisture are reduced, and vegetation growth is inhibited.

The experimental data and monitoring data of this study were obtained under natural conditions, however, it can be difficult in field experiments to ensure that the sample has a single variable such as the maximum soil temperature, minimum soil temperature, and average soil temperature that are exactly the same, and the FTCF changes. However, in this study, the experimental settings, sample layout and sampling were all as far as possible to ensure that the other influencing factors were not too different. In addition, to ensure the reliability of the conclusions, this study was also compared with previous studies [30]. It is hoped that the conclusions of this study will have a certain reference value for the same type of research. In the future, we will continue to experiment and monitor, and the combination of laboratory and field experiments will be strengthened to further verify our conclusions. This study helps to clarify the mechanism underlying the effects of the freezing–thawing cycle on vegetation in alpine regions, and the findings provide new research directions and an important theoretical basis for studying the response of frozen soil ecosystems to climate change.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "conceptualization, Z.M. and B.W.; methodology, Z.M. and B.W.; software, Y.Y.; validation, Z.M., Y.Y. and B.W.; formal analysis, Z.M.; investigation, M.L.; resources, B.W.; data curation, Z.Y.; writing—original draft preparation, Z.M.; writing—review and editing, B.W.; visualization, X.G.; supervision, Y.Y.; project administration, B.W.; funding acquisition, B.W.", please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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