



**Acta Botanica  
Mexicana**

# Bryophyte diversity at a site contaminated by hydrocarbons from abandoned oil wells in Patagonia, Argentina

## Diversidad de briofitas en un sitio contaminado por hidrocarburos de pozos petroleros abandonados en Patagonia, Argentina

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### Abstract:

**Background and Aims:** Bryophytes are distributed worldwide and play a crucial role in disturbed sites, acting as pioneer organisms in ecological successions following disturbances. The objective of this work was to study bryophytes growing in relation to substrates contaminated with crude oil and to analyze their arbuscular mycorrhizal status.

**Methods:** Samples were collected along a northern Patagonian stream located in the steppe (San Carlos de Bariloche, Río Negro, Argentina). The examined specimens were collected along the stream's margins before, during and after the spill from the abandoned oil wells. Bryophyte species were identified based on morpho-anatomical characteristics. The abundance of arbuscular mycorrhizae (AM) in the plants and the number of AM spores in the substrate were also analyzed.

**Key results:** Sixteen bryophyte species were identified; one hornwort, two liverworts and the rest were mosses. Although certain plants were mainly associated with some of the locations studied, bryophyte communities in general did not show significant variations along the pollution gradient, while AM inoculum exhibited variations.

**Conclusions:** This study suggests that certain bryophyte species can tolerate the conditions generated by crude oil contamination and establish themselves in the early stages of environmental succession (e.g. on solidified crude oil beds). These results provide a basis for future ecological restoration efforts.

**Key words:** arbuscular mycorrhizae, hornworts, hydrocarbon tolerance, liverworts, mosses.

### Resumen:

**Antecedentes y Objetivos:** Las briofitas se distribuyen por todo el mundo y juegan un papel crucial en sitios perturbados, actuando como organismos pioneros en sucesiones ecológicas posteriores a disturbios. El objetivo de este trabajo fue estudiar las briofitas que crecen en relación con sustratos contaminados con petróleo crudo y analizar su estado micorrízico arbuscular.

**Métodos:** Las muestras se recolectaron a lo largo de un arroyo de la estepa norpatagónica (San Carlos de Bariloche, Río Negro, Argentina). Los especímenes examinados se recolectaron a lo largo de los márgenes del arroyo antes, durante y después del derrame de pozos petroleros abandonados. Las especies de briofitas fueron identificadas con base en características morfo-anatómicas. También se analizó la abundancia de micorrizas arbusculares (MA) en las plantas y el número de esporas MA en el suelo.

**Resultados clave:** Se identificaron 16 especies de briofitas; una perteneciente a antocerotes, dos a hepáticas y el resto a musgos. Aunque ciertas plantas se asociaron principalmente con algunas de las ubicaciones estudiadas, las comunidades de briofitas en general no mostraron variaciones significativas a lo largo del gradiente de contaminación, mientras que el inóculo MA sí presentó variaciones.

**Conclusiones:** Este estudio sugiere que ciertas especies de briofitas pueden tolerar las condiciones generadas por la contaminación con petróleo crudo y establecerse en las primeras etapas de la sucesión ambiental (por ejemplo, en lechos de petróleo crudo solidificado). Estos resultados proporcionan una base para futuros esfuerzos de restauración ecológica.

**Palabras clave:** antocerotes, hepáticas, micorrizas arbusculares, musgos, tolerancia a hidrocarburos.

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Received: July 3, 2024.

Reviewed: August 22, 2024.

Accepted by Rosario Redonda-Martínez: September 25, 2024.

Published Online first: November 5, 2024.

Published: Acta Botanica Mexicana 131 (2024).

To cite as: Cottet, A. C., M. Soto-Mancilla and N. V. Fernández. 2024. Bryophyte diversity at a site contaminated by hydrocarbons from abandoned oil wells in Patagonia, Argentina. Acta Botanica Mexicana 131: e2379. DOI: <https://doi.org/10.21829/abm131.2024.2379>



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e-ISSN: 2448-7589

## Introduction

The increase in human populations and their consequent demand for space, food, and energy generates impacts on a global-scale environmental. Because these effects are expected to worsen, there is an urgent need to implement effective conservation and remediation measures (Arroyo-Rodríguez et al., 2019). Achieving this requires a comprehensive understanding of ecosystem diversity, as well as the patterns, processes, and mechanisms that determine populations and communities' sustainability in different landscapes (Arroyo-Rodríguez et al., 2019). This issue is not trivial, since, although containment and mitigation measures exist, they are not always sufficient. Given the increasing pressure on global ecosystems due to human activities, it is essential to focus on regions where such impacts are more evident.

Argentina has the fourth largest reserves of crude oil and gas in the world, with more than 70% of the production concentrated in Patagonia, the southern region of the country (Galafassi and Pérez-Roig, 2014). Petroleum is one of the most important economic resources of the country, but its utilisation inherently has harmful effects on the environment which will increase alongside its exploitation (Chen et al., 2019). Oil spills and leaks are recurring events that can affect large areas (Chen et al., 2019). Although in recent decades these incidents have decreased, they are still a significant problem that affects extensive areas, both in marine and terrestrial ecosystems (Chen et al., 2019). According to del Barrio et al. (2020), periodic oil spills have been documented in Northern Patagonia, being one of the three main sources of pollution in this region. Currently, the approach to and treatment of this problem remain largely neglected. Despite the economic importance of the petroleum industry in Patagonia, the environmental repercussions raise serious concerns.

"Abandoned or orphaned wells" refer to sites where oil exploitation has ceased for an indefinite time. Although these wells must present proper sealing in accordance with national regulations (Boletín Nacional de la República Argentina, 1996), this does not always happen. Currently, only in Patagonia, there are more than 2000 abandoned wells (Más energía, 2020), some of which lack adequate sealing (Martín et al., 2008; SAyDS, 2018), including those within

the study area of this work. Areas with a continuous input of fresh contaminants could be considered a serious source of environmental degradation, so these situations deserve urgent attention. This scenario underscores the need to make both economic activities compatible and, consequently, to reduce the environmental impacts inherent to hydrocarbon extraction and transportation. Therefore, it is essential to develop sustainable management and remediation projects. This issue becomes even more critical when considering the continuous contamination that stems from improperly sealed wells, a factor that contributes to long-term environmental degradation.

In light of these environmental challenges, bioassessment has emerged as a crucial tool for monitoring the health of ecosystems. Bioassessment uses organisms to gather information about environment alterations. This criterion is part of the ecological diagnoses that allow us to evaluate the condition and resilience of an environment in response to anthropogenic pressures. In this context, biodiversity and specialisation values are considered reliable indicators (Fredes et al., 2009). This type of approach allows comparison of environmental states by analysing the structural and functional changes of the studied community between the sites, thus assessing the degree of modification or alteration (Fredes et al., 2009). Among the organisms that can be effectively used for bioassessment purposes are bryophytes. Bryophytes are included within three Phyla of the Kingdom Plantae: Anthocerotophyta (hornworts), Bryophyta (mosses) and Marchantiophyta (liverworts) (Söderström et al., 2016). These organisms constitute an ancestral group of land plants with remarkable adaptive versatility, enabling them to thrive in diverse environments. These plants play a crucial role in ecosystems since, along with other organisms, they are pioneers in primary ecological successions, facilitating the germination of woody plants, promoting recolonization by other plant species, preventing erosion and enhancing soil characteristics, such as humidity, organic matter content, and the consolidation of soil profiles (Delgadillo Moya and Cárdenas Soriano, 2009). These poikilohydric organisms absorb water and nutrients directly through their surface, and are capable of incorporating and accumulating significant quantities of toxic substances (e.g. heavy metals). Due to this characteristic, bryophytes react very sensitively to



environmental changes, making them especially suitable as bioindicators of alterations in environmental and climatic conditions (Gradstein, 2002). Among the diverse organisms used for bioassessment, bryophytes stand out due to their sensitivity to environmental changes and their vital role in ecological succession processes.

On the other hand, similar to other representatives of the Plantae Kingdom, bryophytes can form associations with fungi and establish different types of relationships, among which is the mycorrhizal symbiosis (Kowal et al., 2020). Some fungi from the Phylum Mucoromycota have been reported forming arbuscular mycorrhizal (AM) associations with hornworts and liverworts, but not with mosses (Hoysted et al., 2018). The association with mycorrhizal-forming fungi is one of the strategies that allows plants to resist adverse conditions (Brundrett and Tedersoo, 2018). In this scenario, fungal hyphae are generally more physiologically effective at absorbing water and nutrients than rhizoids or vegetative structures of bryophytes (Liepina, 2012). In addition, they are capable of resisting high levels of contamination by forming resistant structures. Therefore, the health and growth of plants are usually improved by AM (Liepina, 2012; Nelson and Shaw, 2019). Given the ecological importance of both bryophytes and AM in natural environments, it is relevant and necessary to deepen our understanding of their relationship, especially in those growing in environments contaminated with hydrocarbons. Moreover, bryophytes are not only important for their ecological roles but also for their associations with mycorrhizal fungi, which enhance their resilience in contaminated environments.

Due to the prolonged presence of hydrocarbons in our study area, we deemed it a good opportunity to research the occurrence and abundance of bryophytes to understand their tolerance to this disturbance. Therefore, the objective of this work was to study bryophytes that grow in relation to substrates contaminated with crude oil and analyse their arbuscular mycorrhizal status.

## Materials and Methods

### Study area

The study area is located 25 km from San Carlos de Bariloche, Río Negro, Argentina, in Northern Patagonia (Fig. 1A). The predominant vegetation of the site is of the arid and semi-ar-

id steppe type, where herbaceous and shrub species are abundant (Cabrera, 1974), with scattered trees of the genus *Nothofagus* Blume on the banks of the stream. In general terms, the Patagonian steppe is characterized by terrain with low mountains, plateaus, and plains. The soils are generally sandy-stony, poor in fine materials and organic matter. The climate is dry and cold, with snow during the winter and frosts almost year-round, as well as very strong winds (Cabrera, 1974).

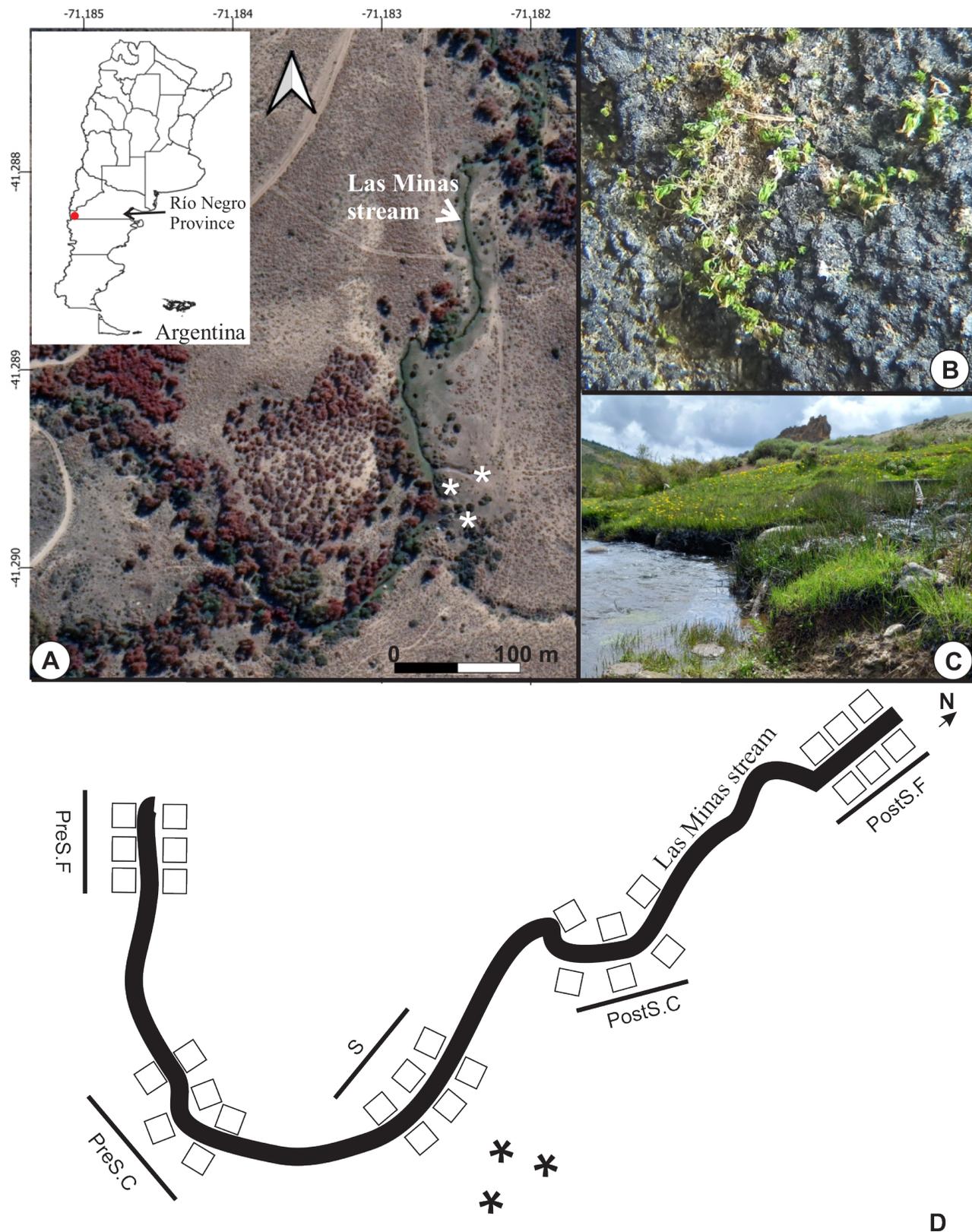
The area has a history of multiple attempts at hydrocarbon extraction activity, which began in 1916-1922 and persisted until 1990 without yielding favourable productive results (Martín et al., 2008; Galafassi and Pérez-Roig, 2014). Currently, in this site there is a natural spring and three abandoned oil wells resulting from exploratory drilling, which were poorly and inadequately sealed. Due to the inclination of the terrain, crude oil flowing from these wells, and not retained by vegetation and soil, reaches the waters of a nearby stream located within the Andean North Patagonian Biosphere Reserve, named Las Minas (Fig. 1A). Although a plan for mitigating the environmental problems caused by these abandoned oil wells was implemented in 2008 (already two decades after the last exploitation attempts), it only focused on containing the crude oil through the installation of containment pipes and tanks. However, due to lack of proper maintenance, these measures were not effective and nowadays the oil continues flowing (Galafassi and Pérez-Roig, 2014; SAYDS, 2018).

### Collection of bryophytes and soil samples

In a first stage, a preliminary survey of bryophytes was conducted in the affected area to evaluate the most suitable sector for carrying out the study. This involved targeted sampling, collecting plants from soil and oiled surfaces within the contaminated site (e.g. Fig. 1B). Since the highest diversity was observed near the stream, samples for the second sampling of the study were taken from its banks to assess the impact of oil on these organisms. In addition to its greater diversity, this area showed a more uniform structure, allowing a more thorough analysis of the impact of flowing oil on these plants (Fig. 1C).

In a second sampling stage, 30 plots of 1 m<sup>2</sup> each were established along both banks of the stream. They





**Figure 1:** Study area and sampling design. A. location of the study area close to San Carlos de Bariloche, Río Negro Province, Argentina; B. moss growing on solidified crude oil; C. general appearance of the spill that reaches the stream, which runs north; D. sampling design. The asterisks symbolize orphaned wells. The squares symbolize the transitional plots. PreS.F=Pre-Spill far from the spill zone; PreS.C=Pre-Spill close to the spill zone; S=the spill zone; PostS.C=Post-Spill close to the spill zone; PostS.F=Post-Spill far from the spill zone.

were distributed across five zones: two located before the spill zone, one of them farther from the spill zone (Pre-Spill far from the spill zone: PreS.F) than the other one (Pre-Spill close to the spill zone: PreS.C); one within the spill zone (S); and the other two after the spill zone, the first one closer to it (Post-Spill close to the spill zone: PostS.C) than the second one (Post-Spill far from the spill zone: PostS.F) (Fig. 1D).

Samples of all the bryophyte morphospecies observed in the field were collected from each plot in labelled paper bags until identification. Each herbarium voucher corresponds to all samples collected in one plot. Additionally, 100 grams of soil from the centre of each plot were also collected in labelled plastic bags. Both types of samples, plants and soil, were dried at room temperature in the laboratory until further treatment.

### Bryophyte identification and mycorrhizal status

For the identification of bryophyte species, the morphology and anatomy of different structures of the gametophyte (e.g., leaf or cells) and, when present, of the sporophyte (e.g. capsule, operculum or calyptra) were carefully examined (Gradstein et al., 2001; Gradstein and Pinheiro da Costa, 2003). General observations (e.g., life form) were conducted using an Olympus SZ30 stereoscopic microscope (Olympus Corp., Tokyo, Japan), while observations on anatomical aspects (e.g., papillae) were made using an Olympus BX50 optical microscope (Olympus Corp., Tokyo, Japan). For the identification and distribution of the taxa found, both general and specific literature was used (Matteri, 1985; Sharp et al., 1994; Ochyra and Matteri, 2001; Gradstein and Pinheiro da Costa, 2003; Cano et al., 2008; Cottet and Messuti, 2023). Species names have been updated according to the TROPICOS database (TROPICOS, 2024).

To evaluate the presence of AM fungi in these plants, three gametophytes from each taxon present in the samples were randomly selected, carefully washed with tapwater, and then stained according to the protocol proposed by Cottet et al. (2018). The stained gametophytes were examined in a sagittal section with a magnifying glass and optical microscope (Olympus BX50, Olympus Corp., Tokyo, Japan) to record the presence of fungal structures (Cottet, 2022), distinguishing between superficial and internal fungal structures. In those plants where the presence of AM

was observed, they were quantified (Cottet and Messuti, 2019) and characterized according to Dickson (2004) and Kowal et al. (2020).

### Characterization of environmental factors

To analyse how environmental conditions influence bryophyte distribution, we evaluated the changes in diverse variables in each of the sampled plots: canopy opening, vegetation coverage, soil pH and electrical conductivity (EC), and number of AM spores in the soil. For each of the sampled plots (Fig. 1D), two photographs were captured with a smartphone (Samsung Galaxy A12, Seoul, South Korea): one of the soil to estimate vegetation cover and another to calculate canopy opening, both of which were analyzed using the program ImageJ (Rasband, 2018).

For the soil samples, pH and electrical conductivity (EC) were measured following conventional measurement protocols in aqueous solution (Smith and Doran, 1997). Arbuscular mycorrhizal spore counting was carried out to estimate the abundance of mycorrhiza inoculum (number of AM spores) in the soil where the bryophytes were growing. For this, AM spores present in the collected soil were extracted using the traditional technique described by Brundrett et al. (1996). Briefly, it consisted of wet sieving with 850  $\mu\text{m}$ , 125  $\mu\text{m}$  and 38  $\mu\text{m}$  meshes, followed by the separation of spores under an optical microscope (Olympus BX50, Corp., Tokyo, Japan).

### Statistical analyses

To evaluate the effect of crude oil on bryophyte richness and on the number of AM spores in the soil where these plants were growing, linear models and linear mixed models were employed. Canopy openness was considered a random factor, and the location of the samples with respect to the spill zone (PreS.F, PreS.C, S, PostS.C and PostS.F) was considered a fixed variable. In addition, non-metric multidimensional scaling (NMDS) and Permutational Multivariate Analysis of Variance (PERMANOVA) analysis were carried out to assess changes in bryophyte communities across the different locations. Analysis of variance were also conducted to evaluate if plot position in stream banks, soil pH and EC, vegetation coverage and canopy openness varied between the different locations. All statistical analyzes were



performed using R software v. 4.3.2 (R Core Team, 2023). The lme4 package was used to estimate parameters, the DHARMA package to check model assumptions (Bartón, 2020), the emmeans package for comparisons (Lenth, 2023), and the vegan and pairwiseAdonis packages for community analyses (Heine, 2023; Oksanen et al., 2023). Comparative analyses were conducted to determine if the variables changed depending on the stream bank in which they were found. Finally, the map of the study area was created using a geographic information system QGIS v. 3.26.3 (QGIS Development Team, 2024).

## Results

### Bryophyte diversity

In the preliminary survey, seven species of bryophytes were identified, comprising six moss species and one liverwort. These bryophytes were found in association with different environmental conditions: four species were exclusively found growing in areas surrounded by fresh crude oil along the margin of the stream; one species occurred between rocks at one of the spill containment sites and had dry crude oil on its gametophytes; and two species were observed

growing inside containment tanks which had dry crude oil deposits (Table 1). No bryophytes were found on the margins of the outcrops of saltwater and fresh oil flowing from the poorly sealed abandoned wells towards the stream. Based on these results, we decided to conduct the second part of this study along the margins of the stream, where the highest richness of bryophytes was observed.

The presence of bryophytes in the 30 plots examined on the margins of the stream was relatively high, as 27 of them exhibited at least one species of bryophyte. Similarly, the number of species found was also notable, with a total of 16 identified species: one hornwort, two liverworts and 13 mosses, which were irregularly distributed across the five studied locations (PreS.F, PreS.C, S, PostS.C and PostS.F) (Appendix). Most of them were previously found during the preliminary survey, with the exception of two species exclusively collected from within the containment tanks (*Ceratodon purpureus* (Hedw.) Brid. and *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr.). *Orthotheciella varia* (Hedw.) Ochyra and *Philonotis krausei* (Müll. Hal.) Broth., both mosses, were the only species present in all five locations. Conversely, certain bryophytes were exclusively found in one of the main lo-

**Table 1:** Bryophyte species found on different substrates during the preliminary survey. (B)=Bryophyta; (M)=Marchantiophyta; BCRU=Herbarium Centro Regional Universitario Bariloche.

Substrate	Species	Examined material
Soil On the margins of the stream, with fresh crude oil on the gametophytes	<i>Drepanocladus longifolius</i> (Wilson ex Mitt.) Broth. ex Paris (B) <i>Marchantia berteriana</i> Lehm. & Lindenb. (M) <i>Philonotis krausei</i> (Müll. Hal.) Broth. (B) <i>Pohlia wahlenbergii</i> (F. Weber & D. Mohr) A.L. Andrews (B)	ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, spill zone, 41°17'22.83"S, 71°10'57.39"W, 23.XI.2022, A. Cottet 1p (BCRU)
Dry crude oil Inside containment tanks, with dry crude oil deposits, without crude oil on the gametophytes	<i>Ceratodon purpureus</i> (Hedw.) Brid. (B) <i>Syntrichia ruralis</i> (Hedw.) F. Weber & D. Mohr (B)	ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, spill zone, 41°17'22.83"S, 71°10'57.39"W, 23.XI.2022, A. Cottet 2p (BCRU)
Between rocks, with dry crude oil on the gametophytes	<i>Didymodon vinealis</i> (Brid.) R.H. Zander (B)	ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, spill zone, 41°17'22.83"S, 71°10'57.39"W, 23.XI.2022, A. Cottet 3p (BCRU)



cations: four species were found only before the spill zone (PreS.F and/or PreS.C), four in the spill zone (S), and two right after it (PostS.C and/or PostS.F; Appendix).

### Environmental factors and bryophyte richness

To analyse how environmental conditions influence bryophyte distribution, we evaluated the changes in different variables in each of the sampled plots (canopy opening, vegetation coverage, soil pH and EC, number of AM spores in the soil, and bryophyte richness), which suppose different contamination levels resulting from hydrocarbons emanating from the abandoned oil wells (Table 2). For pH no significant differences were found between the locations ( $p=0.283$ ). For EC significant differences were observed between the locations, being greatest at the spill zone (S.  $p<0.001$ ). Significant differences in canopy opening were observed between the locations ( $p<0.001$ ). A non-parametric contrast revealed that canopy opening increased considerably towards the affected site, indicating that sites prior to contamination (PreS.F and PreS.C) exhibited lower canopy opening compared to the subsequent sites (S, PostS.C and PostS.F). On the other hand, soil vegetation coverage also showed a significant difference ( $p=0.047$ ). Further contrast analysis revealed this difference to be significant only between the S and PostS.C sites ( $p=0.026$ ), being soil vegetation cover higher at the spill zone (S) than right after it (PostS.C). No differences were found in the comparison of the stream margins for each of the analysed variables ( $p>0.05$  for all variables).

We found a significant decrease in bryophyte richness between sites prior to contamination and those after the spill zone (S, Fig. 2A). Although these differences are signif-

icant, the variable that most influenced bryophyte richness, and that also showed a significant variation along the contamination gradient, was canopy openness (Table 3). In addition, NMDS ordinations (Fig. 2C) and PERMANOVA analysis showed no significant differences in bryophyte communities across the five locations ( $p=0.841$ ).

Regarding the presence of AM fungi, we noted a significant increase in the number of AM spores along the contamination gradient, being higher in the spill zone and beyond (Fig. 2B, Table 2). These significant differences were attributed to two variables: location and canopy openness. When performing correlation analysis with pH, EC and plant cover, it turned out that they did not contribute significantly as variables that explained the difference in the number of spores. We found that *Anthoceros lamellatus* Steph. was the only species with the presence of diagnostic AM structures. The observed colonization corresponded to the *Paris*-type and occupied 28% of the gametophyte. Noticeably, this species was exclusively found in the hydrocarbon spill area.

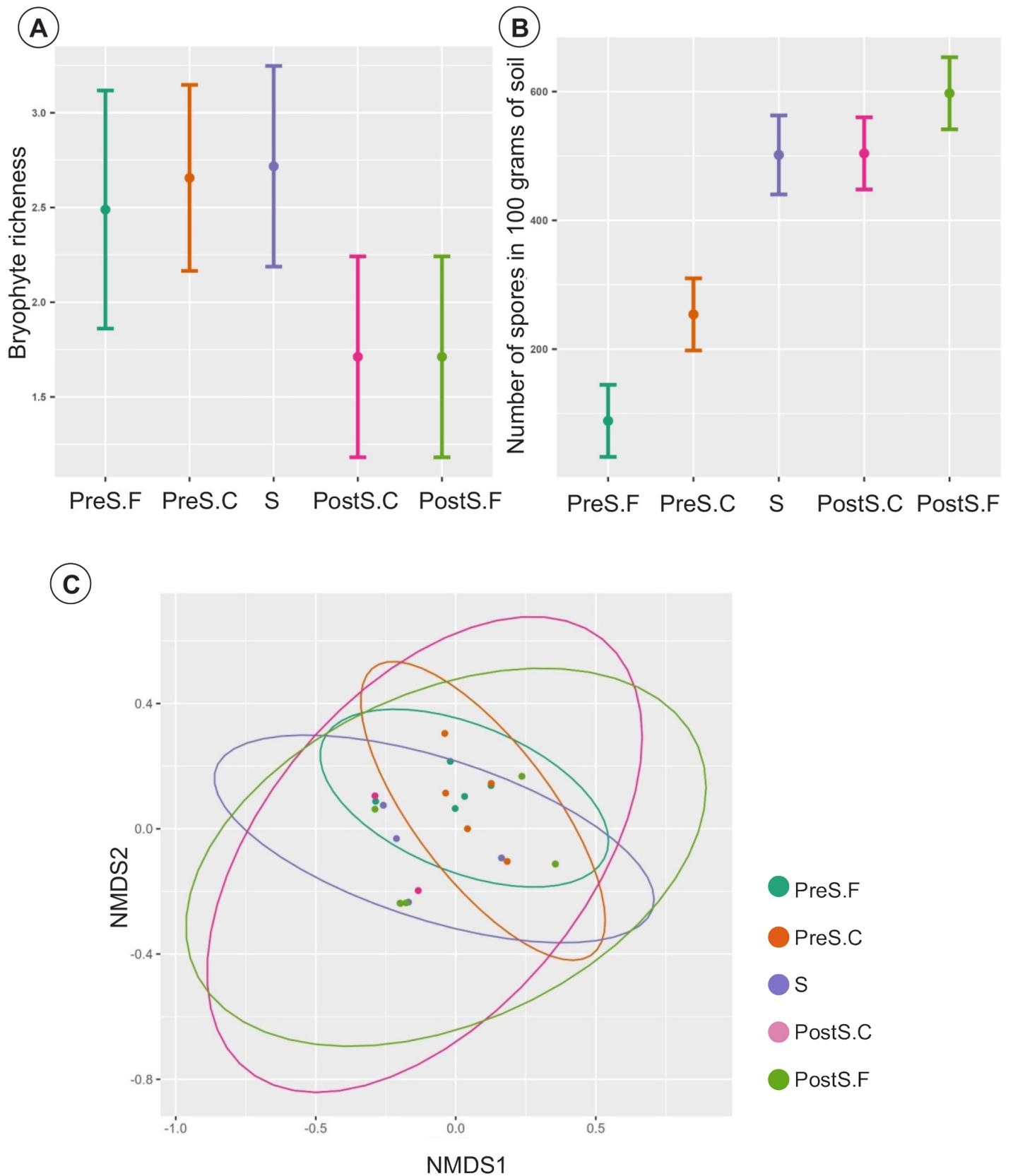
### Discussion

Studies investigating the diversity of bryophytes in crude oil-contaminated environments are scarce. Besides, most of these studies aimed at understanding the impact of hydrocarbons on these plants have been conducted under experimental conditions (e.g. Hutchinson and Freedman, 1978; Storb et al., 2021). One of the novelties of this study is that it was carried out under natural conditions. The exploratory survey revealed that the area immediately surrounding the stream was the most optimal location for assessing the effects of oil on bryophytes. This finding aligns with riparian

**Table 2:** Mean and standard deviation of the environmental variables studied at the different sample locations. EC=electric conductivity, N° AM spores=number of arbuscular mycorrhizal fungi spores per 100 grams of soil, PreS.F=Pre-Spill far from the spill zone, PreS.C=Pre-Spill close to the spill zone, S=hydrocarbon spill zone, PostS.C=Post-Spill close to the spill zone; PostS.F=Post-Spill far from the spill zone. Significance estimator of  $p<0.05$ . Superscript letters in the table denote levels of statistical significance, with different letters representing significantly different groups.

Location	pH	EC ( $\mu$ S)	Canopy openness (%)	Vegetation Coverage (%)	Bryophyte richness	N° AM spores
PreS.F	6.8 $\pm$ 0.3a	65.8 $\pm$ 14.4a	46.1 $\pm$ 8.3a	40.1 $\pm$ 4.7ab	3.2 $\pm$ 0.5a	89 $\pm$ 59a
PreS.C	6.0 $\pm$ 0.3a	85.3 $\pm$ 14.4ac	71.7 $\pm$ 8.3b	42.1 $\pm$ 4.7ab	2.8 $\pm$ 0.5a	254 $\pm$ 59a
S	6.5 $\pm$ 0.3a	109.4 $\pm$ 16.0b	86.7 $\pm$ 9.1b	58.8 $\pm$ 5.1a	2.3 $\pm$ 0.5ab	552 $\pm$ 59b
PostS.C	6.3 $\pm$ 0.3a	85.0 $\pm$ 14.4ac	100.0 $\pm$ 8.3b	31.6 $\pm$ 4.7b	1.3 $\pm$ 0.5b	504 $\pm$ 59b
PostS.F	6.1 $\pm$ 0.3a	73.5 $\pm$ 14.4c	100.0 $\pm$ 8.3b	42.4 $\pm$ 4.7ab	1.3 $\pm$ 0.5b	597 $\pm$ 59b





**Figure 2:** A. bryophyte richness; B. number of spores in the five locations studied; C. NMDS (non-metric multidimensional scaling) ordinations of the bryophyte communities in each of the locations. PreS.F=Pre-Spill far from the spill zone; PreS.C=Pre-Spill close to the spill zone; S=the spill zone; PostS.C=Post-Spill close to the spill zone; PostS.F=Post-Spill far from the spill zone.



**Table 3:** Comparison of the influence of different variables on bryophyte richness. A comparison of variables was performed using an ANOVA with a significance estimator of  $p < 0.05$ . Df=Degrees of Freedom, Sum sq=Sum of Squares, Mean Sq=Mean Square, F value=statistic, p=p-value (level of significance). Level of significance: \*\* =  $p < 0.01$ .

	Df	Sum sq	Mean Sq	F value	p
Canopy opening	1	17.390	17.389	11.551	0.003 **
Location	4	3.416	0.854	0.567	0.689
Canopy opening:location	2	0.874	0.437	0.290	0.751
Residuals	22	33.120	1.505		

sites being biodiversity hotspots in arid and semi-arid environments (Tockner and Stanford, 2002).

During the second sampling, 16 species were found in relation to different environmental characteristics, which contributes to our understanding of bryophyte responses in environments contaminated with crude oil. In addition to identifying species capable of tolerating contamination, we seek to contribute to the comprehension of how bryophyte communities and their mycorrhizal symbionts vary in natural environments. Therefore, this approach allowed us to assess not only the bryophyte diversity in this particular environment, but also their response to this disturbance. The high number of plots containing bryophytes, despite low vegetation cover and high exposure, underscored their ubiquity and highlights their potential as excellent bioindicators (Gradstein, 2002), particularly for this system.

Among the 16 species surveyed, the majority were mosses (13, 81.3%), followed by liverworts (2, 12.5%) and hornworts (1, 6.2%). These findings are not surprising given that the proportions of these Phyla are typical on a global scale (Estébanez Pérez et al., 2011). In Argentine Patagonia, bryophyte diversity surveys have been primarily focused on forested and humid environments in the western region (Cottet, 2023), while records from the eastern and drier portion of Patagonia are scarce and fragmented, mostly appearing in taxonomic surveys rather than analyses of communities' responses to disturbances, as presented here. The found species aligned with expectations and previous observations made for riparian steppe environments (Cottet, 2022).

Regarding the species collected, some, such as *Philonotis krausei* or *Pohlia wahlenbergii* (F. Weber & D. Mohr) A.L. Andrews, are typically found in sites subjected to anthropic disturbances (Sharp et al., 1994). Some oth-

ers, such as *Drepanocladus longifolius* (Wilson ex Mitt.) Broth. ex Paris and *Orthotheciella varia*, have wide morphological variability that enables them to thrive in diverse environmental conditions (Matteri, 1985). This is likely the reason why these four species exhibited a broad distribution across the five studied locations. Consequently, they could serve as promising candidates for ecological restoration trials, since they demonstrate resilience not only to changes in canopy opening but also to pollution. Conversely, species like *Amblystegium humile* (P. Beauv.) Crundw., *Bryoerythrophyllum recurvirostris* (Hedw.) P.C. Chen, *Didymodon vinealis* (Brid.) R.H. Zander, and *Plagiothecium* sp. were exclusively found in sites where crude oil was present, suggesting opportunistic colonization of these altered conditions rather than being integral components of the typical bryoflora of the area. Other species such as *Anthoceros lamellatus* Steph., *Lophocolea bidentata* (L.) Dumort. and *Marchantia berteriana* Lehm. & Lindenb. were absent in post-spill sites, characterized by 100% canopy openness, possibly due to their sensitivity to direct sunlight exposure (*A. lamellatus* and *M. berteriana*), lack of tolerance to oil contamination (*L. bidentata*), or both factors. Another of the surveyed species was *Acrocladium auriculatum* (Mont.) Mitt., a very common post-fire Patagonian species found in both steppes and forests, generally associated with fallen branches and trunks (Ochyra and Matteri, 2001). Despite its tolerance to disturbance, its reduced occurrence between pre-contamination and contaminated sites suggests it may have little tolerance to the presence of crude oil, thus making it a suitable potential candidate for bioindicator of changes in environmental conditions. The same phenomenon may also be occurring with species as *Anomobryum polysetum* A.J. Shaw, *Dicranel-*



*la vaginata* (Hook.) Spruce, *Lophocolea bidentata* or *Philonotis polymorpha* (Müll. Hal.) Kindb.

Canopy opening varied significantly along the stream mainly due to variations in vegetation structure at the site. Since bryophyte communities are directly affected by solar radiation (Wang et al., 2022), the obtained results, where canopy opening emerged as one of the primary factors explaining the decline in richness between locations, made sense. These findings suggest that plants are naturally distributed in response to different stress factors, and that even in areas with the greatest stress (total sunlight exposure and presence of crude oil) they still thrive. On the other hand, community analysis showed that species patches do not vary significantly among the different locations, probably due to low richness and the shared presence of some species such as *P. krausei*. In this place, restoration plans based on bryophytes would initially be recommended focusing on species found in four or five locations, being possible to use portions of patches from unaffected areas as source of inoculum.

The number of AM spores in soil serves as a proxy for assessing the inoculum pressure within an environment, enabling the evaluation of whether bryophytes remain uncolonized due to a lack of inoculum or because they are just non-mycorrhizal under the studied conditions. The spore count varied significantly among the locations, with higher counts observed in contaminated environments. This trend may stem from one of the strategies employed by AM fungi, which involves the formation of spores (considered as resistance structures) under stressful conditions, such as the presence of crude oil. Notably, mycorrhizal colonization was only observed in one of the identified species (*Anthoceros lamellatus*), occurring at a site characterized by high oil content and intermediate inoculum pressure (S site). This observation aligns with what was proposed by different authors who suggested that AM colonization is contingent upon the specific plant and fungal taxa involved rather than solely on the quantity of inoculum present in the substrate (Ligrone et al., 2007; Desirò et al., 2013; Hoysted et al., 2018). The absence of AM colonization in bryophytes within locations characterized by high spore counts (PostS.C and PostS.F) further reinforces this hypothesis.

Some of the strengths of this study lie in its contribution to the characterization of bryophyte communities in arid and semi-arid environments of South America, as well as in assessing how a significant disturbance such as hydrocarbon contamination can affect these plants and their mycorrhizas. Thanks to this survey, it can be inferred that certain species of bryophytes may show tolerance to the different conditions resulting from crude oil contamination, enabling their establishment in early stages of succession (e.g. when growing on beds of solidified crude oil), thus emerging as promising candidates for carrying out restoration efforts. Therefore, these findings provide the bases for future ecological restoration activities and trials conducted under controlled conditions, such as growth chambers and nurseries, as well as *in situ* field conditions.

## Author contributions

ACC collected and identified the examined material. ACC and MSM performed the spore counts. MSM conducted the statistical analyses. All authors participated in the research, sampling design, as well as in the preparation and finalization of the manuscript.

## Funding

This work was funded by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Buenos Aires, Argentina), the Universidad Nacional del Comahue (UNComahue, San Carlos de Bariloche, Argentina), and by the Proyecto de Investigación Científica y Tecnológica (PICT2019-0826, San Carlos de Bariloche, Argentina).

## Acknowledgements

We are grateful to the Crespo family, owners of the place where this study was carried out, for allowing us to carry out the sampling. Sincere thanks are also extended to the anonymous reviewers.

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**Appendix:** Bryophyte species recorded at the five studied locations. PreS.F=Pre-Spill far from the spill zone, PreS.C=Pre-Spill close to the spill zone, S=hydrocarbon spill zone, PostS.C=Post-Spill close to the spill zone; PostS.F=Post-Spill far from the spill zone, +=presence of the species at that location. BCRU=Herbarium Centro Regional Universitario Bariloche.

Bryophyte taxon	Location					Material examined
	PreS.F	PreS.C	S	PostS.C	PostS.F	
<b>Anthocerotophyta</b>						
<i>Anthoceros lamellatus</i> Steph.			+			ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, spill zone, 41°17'22.83"S, 71°10'57.39"W, 15.VI.2023, A. <i>Cottet 17p</i> (BCRU).
<b>Bryophyta</b>						
<i>Acrocladium auriculatum</i> (Mont.) Mitt.	+	+		+		ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill far from the spill zone, 41°17'25.13"S, 71°11'0.37"W, 15.VI.2023, A. <i>Cottet 7p</i> (BCRU); pre-spill close to the spill zone, 15.VI.2023, 41°17'24.19"S, 71°10'58.41"W, A. <i>Cottet 11p</i> (BCRU); post-spill close to the spill zone, 41°17'21.76"S, 71°10'57.61"W, A. <i>Cottet 23p</i> (BCRU).
<i>Amblystegium humile</i> (P. Beauv.) Crundw.				+	+	ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, post-spill close to the spill zone, 41°17'21.76"S, 71°10'57.61"W, A. <i>Cottet 22p</i> (BCRU); post-spill far from the spill zone, 41°17'19.94"S, 71°10'56.92"W, 15.VI.2023, A. <i>Cottet 29p</i> (BCRU), <i>30p</i> (BCRU).
<i>Anomobryum polysetum</i> A.J. Shaw		+				ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill close to the spill zone, 15.VI.2023, 41°17'24.19"S, 71°10'58.41"W, A. <i>Cottet 12p</i> (BCRU).
<i>Bryoerythrophyllum recurvirostrum</i> (Hedw.) P.C. Chen			+			ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, spill zone, 41°17'22.83"S, 71°10'57.39"W, 15.VI.2023, A. <i>Cottet 20p</i> (BCRU).
<i>Dicranella vaginata</i> (Hook.) Spruce	+	+				ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill far from the spill zone, 41°17'25.13"S, 71°11'0.37"W, 15.VI.2023, A. <i>Cottet 4p</i> (BCRU).
<i>Didymodon vinealis</i> (Brid.) R.H. Zander			+			ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, spill zone, 41°17'22.83"S, 71°10'57.39"W, 15.VI.2023, A. <i>Cottet 18p</i> (BCRU)

## Appendix: Continuation.

Bryophyte taxon	Location					Material examined
	PreS.F	PreS.C	S	PostS.C	PostS.F	
<i>Drepanocladus longifolius</i> (Wilson ex Mitt.) Broth. ex Paris	+	+	+		+	ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill far from the spill zone, 41°17'25.13"S, 71°11'0.37"W, 15.VI.2023, A. Cottet 6p (BCRU); pre-spill close to the spill zone, 15.VI.2023, 41°17'24.19"S, 71°10'58.41"W, A. Cottet 13p (BCRU), 14p (BCRU); spill zone, 41°17'22.83"S, 71°10'57.39"W, 15.VI.2023, A. Cottet 20p (BCRU); post-spill far from the spill zone, 41°17'19.94"S, 71°10'56.92"W, 15.VI.2023, A. Cottet 32p (BCRU), 33p (BCRU).
<i>Orthotheciella varia</i> (Hedw.) Ochyra	+	+	+	+	+	ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill far from the spill zone, 41°17'25.13"S, 71°11'0.37"W, 15.VI.2023, A. Cottet 6p (BCRU), 7p (BCRU), 9p (BCRU); pre-spill close to the spill zone, 15.VI.2023, 41°17'24.19"S, 71°10'58.41"W, A. Cottet 11p (BCRU), 13p (BCRU); spill zone, 41°17'22.83"S, 71°10'57.39"W, 15.VI.2023, A. Cottet 17p (BCRU), 18p (BCRU); post-spill close to the spill zone, 41°17'21.76"S, 71°10'57.61"W, A. Cottet 25p (BCRU), 27p (BCRU); post-spill far from the spill zone, 41°17'19.94"S, 71°10'56.92"W, 15.VI.2023, A. Cottet 29p (BCRU), 33p (BCRU).
<i>Philonotis krausei</i> (Müll. Hal.) Broth.	+	+	+	+	+	ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill far from the spill zone, 41°17'25.13"S, 71°11'0.37"W, 15.VI.2023, A. Cottet 4p (BCRU), 5p (BCRU), 7p (BCRU), 8p (BCRU), 9p (BCRU); pre-spill close to the spill zone, 15.VI.2023, 41°17'24.19"S, 71°10'58.41"W, A. Cottet 10p (BCRU), 11p (BCRU), 12p (BCRU), 13p (BCRU), 14p (BCRU), 15p (BCRU); spill zone, 41°17'22.83"S, 71°10'57.39"W, 15.VI.2023, A. Cottet 19p (BCRU), 21p (BCRU); post-spill close to the spill zone, 41°17'21.76"S, 71°10'57.61"W, A. Cottet 27p (BCRU); post-spill far from the spill zone, 41°17'19.94"S, 71°10'56.92"W, 15.VI.2023, A. Cottet 28p (BCRU), 33p (BCRU).
<i>Philonotis polymorpha</i> (Müll. Hal.) Kindb.	+					ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill far from the spill zone, 41°17'25.13"S, 71°11'0.37"W, 15.VI.2023, A. Cottet 4p (BCRU), 5p (BCRU).
<i>Plagiothecium</i> sp.			+			ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, spill zone, 41°17'22.83"S, 71°10'57.39"W, 15.VI.2023, A. Cottet 19p (BCRU).



## Appendix: Continuation.

Bryophyte taxon	Location					Material examined
	PreS.F	PreS.C	S	PostS.C	PostS.F	
<i>Pohlia wahlenbergii</i> (F. Weber & D. Mohr) A.L. Andrews		+	+	+		ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill close to the spill zone, 15.VI.2023, 41°17'24.19"S, 71°10'58.41"W, A. Cottet 12p (BCRU), 14p (BCRU); spill zone, 41°17'22.83"S, 71°10'57.39"W, 15.VI.2023, A. Cottet 17p (BCRU), 18p (BCRU)
<i>Sanionia uncinata</i> (Hedw.) Loeske				+		ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, post-spill close to the spill zone, 41°17'21.76"S, 71°10'57.61"W, A. Cottet 22p (BCRU).
<b>Marchantiophyta</b>						
<i>Lophocolea bidentata</i> (L.) Dumort.	+					ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill far from the spill zone, 41°17'25.13"S, 71°11'0.37"W, 15.VI.2023, A. Cottet 7p (BCRU), 9p (BCRU); post-spill close to the spill zone, 41°17'21.76"S, 71°10'57.61"W, A. Cottet 23p (BCRU).
<i>Marchantia berteriana</i> Lehm. & Lindenb.	+	+	+			ARGENTINA. Province Río Negro, San Carlos de Bariloche, Las Minas stream, pre-spill far from the spill zone, 41°17'25.13"S, 71°11'0.37"W, 15.VI.2023, A. Cottet 4p (BCRU), 5p (BCRU), 6p (BCRU), 7p (BCRU); pre-spill close to the spill zone, 15.VI.2023, 41°17'24.19"S, 71°10'58.41"W, A. Cottet 12p (BCRU), 13p (BCRU); spill zone, 41°17'22.83"S, 71°10'57.39"W, 15.VI.2023, A. Cottet 16p (BCRU), 17p (BCRU), 18p (BCRU); post-spill close to the spill zone, 41°17'21.76"S, 71°10'57.61"W, A. Cottet 25p (BCRU); post-spill far from the spill zone, 41°17'19.94"S, 71°10'56.92"W, 15.VI.2023, A. Cottet 29p (BCRU).

