UNIVERSITÉ DU QUÉBEC À MONTRÉAL

# LARGEUR DE LA NICHE ALIMENTAIRE DE DAPHNIA PULICARIA DANS UNE MINE ABANDONNÉE ET SUBMERGÉE ET RELATIONS AVEC LES PARAMÈTRES ABIOTIQUES

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# LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

ANOVA : Analysis of Variance

CERMO-FC : Centre excellence en recherche sur les maladies orphelines – Fondation Courtois

CID : carbon inorganique dissout

COD : carbone organique dissout

Cu : copper

*D. pulicaria* : *Daphnia pulicaria*

db-RDA : distance-based Redundancy Analysis

DIC : dissolved inorganic carbon

DVM : diel vertical migration

DO : dissolved oxygen

DOC : dissolved organic carbon

Fe : iron

GRIL : Groupe de Recherche Interuniversitaire en Limnologie

ICP-QQQ : Inductively Coupled Plasma Triple Quadrupole Mass Spectrometer

LAE-ISE : Laboratoire d’Analyses Environnementales de l’Institut des Sciences de l’Environnement

MB : Mine Blackburn

MOP : matière organique particulaire

$NH_x$ : ammonia and ammonium

$NO_2^-$ : nitrites

$NO_3^-$ : nitrates

PCoA : Principal Coordinate Analysis

PERMANOVA : Permutational Multivariate Analysis of Variance

PIB : produit intérieur brut

POM : particulate organic matter

UQAM : Université du Québec à Montréal

ΣREE : sum of rare earth elements

# RÉSUMÉ

Peu d'études ont porté sur l'écologie des réseaux trophiques dans les lacs miniers méromictiques, malgré l'abondance de ces plans d'eau abandonnés issus de l'exploitation minière, qui présentent aujourd'hui des enjeux de gestion et de restauration. Dans cette étude, nous avons examiné la niche trophique d'une population de *Daphnia pulicaria* vivant dans un lac minier submergé de type méromictique situé à Val-des-Monts, au Québec, Canada.

Les objectifs spécifiques de cette étude étaient les suivants : (1) évaluer comment la diversité, la composition des communautés et les signatures isotopiques ($\delta^{13}C$ et $\delta^{15}N$) des communautés microbiennes servant de ressources alimentaires à *Daphnia pulicaria* variaient en réponse aux facteurs abiotiques le long du gradient de profondeur et sur de courtes périodes de temps, tant diurnes qu'au cours du début de l'été ; (2) examiner l'amplitude de la niche trophique de *D. pulicaria* en lien avec les variations potentielles des ressources alimentaires selon la profondeur ; et (3) étudier les schémas de migration verticale journalière de *D. pulicaria* en lien avec sa niche trophique.

Nous avons formulé l'hypothèse selon laquelle les variations abiotiques selon la profondeur, entre le jour et la nuit, et au cours du début de l'été, influenceraient significativement la diversité et la composition des communautés microbiennes utilisées comme ressources alimentaires par *D. pulicaria*, ainsi que les signatures isotopiques du carbone et de l'azote de la matière organique particulaire. De plus, nous nous attendions à ce qu'en l'absence de prédateurs visuels et en réponse aux conditions abiotiques contraignantes, *D. pulicaria* limiterait ses déplacements verticaux et présenterait ainsi des signatures isotopiques $\delta^{13}C$ et $\delta^{15}N$ spécifiques à la profondeur.

En nous concentrant sur *D. pulicaria*, un consommateur primaire clé, nous apportons de nouvelles connaissances sur les dynamiques écologiques des lacs miniers méromictiques, où des conditions hostiles telles que l'anoxie complique la vie du zooplancton. À notre connaissance, il s'agit de la première étude évaluant comment les paramètres abiotiques influencent la niche trophique de *D. pulicaria* dans un lac minier méromictique, en mettant en évidence l'impact des gradients environnementaux sur ses ressources alimentaires, ainsi que sa répartition verticale.

Bien que la diversité des ressources alimentaires soit restée relativement stable avec la profondeur, des changements dans la composition des communautés microbiennes et les signatures isotopiques $\delta^{13}C$ et $\delta^{15}N$ de la matière organique particulaire ont révélé une transition entre un carbone d'origine photosynthétique en surface et un carbone transformé par voie microbienne en profondeur. La niche trophique de *D. pulicaria*, reflétée par les isotopes stables du carbone, était plus large dans le mixolimnion, probablement en raison d'une plus grande disponibilité des ressources dans cette couche. Comme prévu, *D. pulicaria* n'a pas montré de migration verticale journalière, probablement en raison des conditions anoxiques dans la monimolimnion et de l'absence de prédation par les poissons. Les individus sont restés majoritairement au-dessus de la chimocline.

Ces résultats mettent en lumière la complexité des interactions entre les facteurs abiotiques, les processus microbiens et les dynamiques trophiques du zooplancton dans les lacs de mines méromictiques. Des recherches futures pourraient explorer les variations saisonnières et interannuelles de la dynamique trophique de *D. pulicaria* afin de déterminer si ces tendances persistent dans un contexte de changements abiotiques. Par ailleurs, l'intégration d'analyses isotopiques spécifiques aux composés et d'approches métagénomiques pourrait affiner notre compréhension du rôle des microbes dans le cycle du carbone et les interactions trophiques au sein de ces écosystèmes.

**Mots-clés** : 18S/16S rRNA, $\delta^{13}C$, $\delta^{15}N$, lac minier, *Daphnia*, communautés microbiennes

# ABSTRACT

Few studies have been conducted on the food web ecology of basal trophic levels in meromictic mining lakes. This is despite the abundance of abandoned, mining-impacted water bodies scattered across the landscape that need to be managed and restored. In our study, we investigated the basal microbial communities acting as dietary resource and the dietary niche of a *Daphnia pulicaria* population across depth, time, and abiotic factors in a meromictic mining pit lake. The specific objectives of this study were as follows : to (1) assess how the diversity, community composition, and isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of microbial communities serving as dietary resources for *Daphnia pulicaria* varied in response to abiotic factors along the depth gradient and over short temporal scales, including diel and early summer changes; (2) examine the trophic niche breadth of *D. pulicaria* in relation to differences in available dietary resources across the depth gradient; and (3) investigate patterns of diel vertical migration of *D. pulicaria* in relation to its trophic niche.

We hypothesized that abiotic variations along the depth gradient, between day and night periods, and over the course of early summer would significantly influence both the diversity and composition of microbial communities serving as dietary resources for *Daphnia pulicaria*, as well as the carbon and nitrogen isotopic signatures of particulate organic matter. Additionally, we expected that, in the absence of visual predators and under abiotic constraints, *D. pulicaria* would restrict its vertical movement and display depth-specific $\delta^{13}C$ and $\delta^{15}N$ signatures. By focusing on *D. pulicaria*, a key primary consumer, we offer new insights into the ecological dynamics of meromictic mining lakes, where hostile conditions such ass anoxia challenge zooplanktonic life. To our knowledge, this is the first study to evaluate how abiotic parameters shape the trophic niche of *Daphnia pulicaria* in a meromictic mining lake, offering new insights into food-web dynamics and the influence of environmental gradients on its dietary resources and vertical distribution.

While the diversity of food resources remained stable across depths, depth-related shifts in microbial community composition and the $\delta^{13}C$ and $\delta^{15}N$ signatures of POM revealed a transition from surface-derived photosynthetic carbon to deeper carbon pools influenced by microbial processes. At these depths, the dominance of green sulfur bacteria (e.g., *Chlorobium* at 6 meters), methanotrophic activity at the chemocline, and the use of respired $CO_2$ in chemosynthetic pathways could all contribute to the increasingly depleted $\delta^{13}C$ signatures observed. *D. pulicaria*'s trophic niche, as indicated by stable carbon isotopes, was broader in the mixolimnion, likely due to higher availability. As anticipated, *D. pulicaria* did not exhibit diel vertical migration, likely due to anoxic conditions in the monimolimnion and the absence of fish predation. As a result, individuals remained predominantly above the chemocline.

These findings underscore the complexity of interactions between abiotic factors, microbial processes, and zooplankton trophic dynamics in meromictic mining lakes. Future research could focus on seasonal and interannual variations in *D. pulicaria* trophic dynamics to determine whether these patterns persist under changing abiotic conditions. Additionally, integrating compound-specific isotope analyses and metagenomic approaches could enhance our understanding of the role of microbes in carbon cycling and trophic interactions in these ecosystems.

Keywords : 18S/16S rRNA, $\delta^{13}C$, $\delta^{15}N$, mining pit lake, *Daphnia*, microbial communities

# INTRODUCTION

L'industrie minière est un pilier de l'économie canadienne, tant par la richesse qu'elle génère que par les emplois qu'elle crée, directement et indirectement (Marshall, 2021). En 2020, elle a contribué à environ 5 % du PIB canadien (Marshall, 2021). Le Québec, dont la géologie favorise la formation de gisements métalliques, abrite le plus grand nombre de mines de métal au pays, avec 24 des 70 mines actives au Canada en 2020 (Marshall, 2021). L'exploitation minière y a débuté en 1823 avec l'extraction de l'or, suivie par celle du phosphate dans les années 1870 (Cranstone, 2002). Un tournant s'opère en 1920 avec la création de départeoments d'exploration au sein des compagnies minières, intégrant des géologues et des prospecteurs. Dans les années 1950, l'industrie se diversifie grâce aux avancées en géophysique et en exploration, facilitant la découverte de nouveaux gisements de nickel, cuivre, zinc, plomb et fer (Cranstone, 2002). Avant l'instauration de la Loi sur les mines du Québec en 1991, les mines étaient souvent abandonnées une fois exploitées, sans travaux de restauration. En 2024, on comptait 346 sites miniers abandonnés au Québec et environ 10 000 à l'échelle du Canada, définis par le ministère des Ressources naturelles et des Forêts (MRNF) comme des sites où l'activité minière a cessé définitivement et dont aucun responsable n'est connu ou solvable (MRNF, 2024; Natural Resources Canada, 2025). Face au coût élevé de la restauration, le gouvernement impose désormais aux compagnies minières de soumettre un plan de réhabilitation avant l'obtention d'un bail minier.

Lorsqu'une mine est abandonnée et que le drainage des eaux cesse, les fosses minières s'inondent progressivement par la remontée des eaux souterraines, formant ainsi des lacs miniers (Nixdorf et al., 2005; Wu et al., 2004). En raison de leur faible rapport surface/profondeur et de leurs berges abruptes, ces lacs sont plus enclins à devenir méromictiques que les lacs naturels (Boehrer & Schultze, 2006). Leur stratification est marquée par un mixolimnion bien oxygéné en surface et un monimolimnion anoxique en profondeur, séparés par une chimiocline riche en sulfures d'hydrogène et en métaux dissous (Gulati et al., 2017). Cette structure limite le mélange vertical et favorise l'accumulation de métaux et de métalloïdes dans les couches profondes (von Gunten et al., 2019). L'activité minière altère également la qualité des eaux de surface et souterraines (Calugaru et al., 2016, 2017; Zverev et al., 2023). Le drainage minier peut être acide, neutre ou basique (Calugaru et al., 2016; Nordstrom et al., 2015). Le drainage neutre contaminé (DNC), bien que moins étudié que le drainage minier acide, demeure préoccupant pour l'environnement. Il présente un pH entre 6 et 9 et contient des sulfates ainsi que des métaux dissous issus de l'oxydation des sulfures (Nordstrom et al., 2015). Le nickel et le zinc, solubles à pH neutre, peuvent y être présents en concentrations élevées (Calugaru et al., 2016, 2017). Ces contaminants affectent la biodiversité en modifiant les structures communautaires et en réduisant la richesse spécifique (Ferrari et al., 2015; Hogsden et al., 2013). Les lacs miniers sont progressivement colonisés par des microorganismes issus des surfaces minérales, des roches et des sols des galeries, ainsi que des eaux environnantes (Lhoste et al., 2023). Le mixolimnion est majoritairement dominé par les algues et le phytoplancton (Gulati et al., 2017; Murtaugh, 1985), tandis que la chimiocline abrite une communauté microbienne dense, comprenant des bactéries sulfureuses vertes et pourpres capables de photosynthèse anoxygénique (Camacho et al., 2001; Gulati et al., 2017). Ces environnements hostiles hébergent aussi du zooplancton, comme des cladocères et des rotifères (Camacho et al., 2001; Coetzee, 1981).

Dans le cadre de cette étude, nous nous intéressons au site de la mine Blackburn, située à 5 km au nord-est du village de Perkins, dans la municipalité de Val-des-Monts, Québec. Exploitée dès les années 1870 par les frères Blackburn, cette mine a d’abord produit du phosphate avant de se tourner vers le mica à partir de la fin des années 1880. En 1891, elle devient l’une des premières mines canadiennes à fournir du mica pour l’industrie électrique et emploie jusqu’à 120 mineurs et 800 ouvrières dans un atelier de découpe à Ottawa. L’exploitation cesse en 1942, laissant la mine à l’abandon sans travaux de restauration. Aujourd’hui, la mine Blackburn est totalement submergée et sous la responsabilité de la province du Québec et de la municipalité de Val-des-Monts. Une clôture délimite l’ouverture du cratère à ciel ouvert, et le site, désormais peu fréquenté, a vu le retour d’une végétation abondante ainsi que de plusieurs espèces animales, notamment des amphibiens et des rongeurs. Ce site minier génère un drainage neutre contaminé, à l’origine du lac méromictique dont le pH est proche de la neutralité. De précédentes campagnes d’échantillonnage réalisées par Élise Lhoste (doctorante impliquée dans le projet des mines abandonnées) et/ou moi-même ont permis de décrire la stratification et les principales caractéristiques abiotiques présentées ci-dessous (données non publiées). Le mixolimnion, oxique et relativement translucide, s’étend jusqu’à environ 2,5 mètres de profondeur. La chimiocline, située entre 2,5 et 4,5 mètres selon le niveau de l’eau, est marquée par un nuage de sulfure d’hydrogène réduisant fortement la visibilité. En dessous, le monimolimnion, qui s’étend jusqu’à 27 mètres, est caractérisé par une absence d’oxygène, une faible concentration en matière organique et une turbidité extrême. La seule espèce de zooplancton présente dans les eaux de ce lac est *Daphnia pulicaria*, qui n’est prédatée que par les larves de *Chaoborus* spp.

Des études antérieures sur les communautés microbiennes dans les lacs méromictiques ont révélé des schémas verticaux distincts dans la composition et la structure des assemblages procaryotes et eucaryotes, façonnés par des gradients environnementaux en fonction de la profondeur, tels que la concentration en oxygène et la disponibilité lumineuse. Dans un lac de montagne méromictique, Oikonomou et al. (2014) ont montré que les protistes mixotrophes jouaient un rôle écologique clé par une activité de broutage importante dans le mixolimnion oxique, tandis que la pression de broutage à la chimiocline avait peu d'influence sur l'abondance procaryote et était négligeable dans les couches anoxiques. Une étude subséquente des mêmes auteurs (Oikonomou et al., 2015) a révélé que les communautés de protistes du mixolimnion subissaient les plus grands changements saisonniers, que la diversité atteignait un pic à la chimiocline suboxique au printemps et en été, et que les assemblages du monimolimnion demeuraient les plus stables au cours de l'année. Dans une ancienne mine de soufre méromictique, Wilk-Woźniak & Żurek (2006) ont observé que la communauté phytoplanctonique était composée d'un nombre restreint d'espèces – incluant cyanoprokaryotes, chrysophytes, diatomées, eugléniens, dinoflagellés, cryptophytes et chlorophytes – les diatomées étant même présentes dans le monimolimnion anoxique. Krasnova et al. (2015) ont également noté que, dans les couches intermédiaires entre les eaux aérobies et anoxiques, des bactéries vertes et pourpres sulfureuses ainsi que des organismes eucaryotes tolérants à l'anoxie, souvent mixotrophes, étaient présents. De plus, Edberg et al. (2012) ont montré que, dans une ancienne mine d'uranium à ciel ouvert, la transition d'un mixolimnion oxique à un monimolimnion anoxique, alcalin et enrichi en métaux et sulfures, s'accompagnait d'une transformation marquée de la communauté bactérienne. Alors que le mixolimnion présentait une composition semblable à celle d'autres systèmes d'eau douce tempérés, le monimolimnion était caractérisé par une plus grande diversité bactérienne dominée par des bactéries phototrophes sulfureuses vertes et des OTUs affiliés aux réducteurs de fer ferrique et de sulfate. Enfin, Andrei et al. (2015) ont observé que, dans un lac hypersalin méromictique, la diversité bactérienne diminuait de la surface jusqu'à la chimiocline, puis augmentait de nouveau dans les couches plus profondes. Collectivement, ces études soulignent comment les gradients environnementaux verticaux influencent la composition et la diversité des communautés microbiennes dans les lacs méromictiques, affectant ainsi la structure des ressources alimentaires basales. Cependant, la recherche microbienne dans ces systèmes demeure souvent fragmentée, se concentrant soit sur les procaryotes, soit sur les eucaryotes, et intègre rarement les échelles spatiales et temporelles fines ou les dynamiques journalières. De plus, l'influence de ces patrons microbiens liés à la profondeur sur les niveaux trophiques supérieurs, tels que le zooplancton, reste peu explorée.

*Daphnia* est un genre de petit crustacé qui comprend plus de 1000 espèces identifiées à ce jour (Ebert, 2022). Les daphnies jouent un rôle important dans l'équilibre des écosystèmes d'eau douce (Carpenter et al., 1987), puisque ces brouteurs généralistes sont les principaux consommateurs primaires d'algues, de bactéries et de protozoaires, et sont les proies de poissons et de divers prédateurs invertébrés (Ebert, 2022; Miner et al., 2012; Tessier et al., 2000). En raison de leur rôle central dans les réseaux trophiques aquatiques, les comportements migratoires des daphnies ont fait l'objet de nombreuses études visant à comprendre les facteurs qui influencent leur répartition verticale dans la colonne d'eau. De nombreuses études ont montré que le principal facteur déterminant la migration verticale nycthémérale (DVM) de *Daphnia* est la présence de poissons (Ringelberg, 1991; Zaret & Suffern, 1976). Dans un lac méromictique où les poissons étaient présents, Boronat & Miracle (1997) ont observé que *Daphnia* présentait une migration de faible amplitude, reflétant un compromis entre l'évitement des prédateurs et l'optimisation de l'apport alimentaire. À l'inverse, d'autres travaux suggèrent que des conditions alimentaires défavorables en profondeur et l'absence de poissons peuvent entraîner une migration réduite ou absente (Beklioglu et al., 2008; Dini & Carpenter, 1992), laissant supposer que la disponibilité alimentaire pourrait devenir le facteur prédominant dans les lacs de mines méromictiques dépourvus de poissons. En effet, Zadereev & Tolomeyev (2007) ont rapporté qu'en l'absence de prédateurs visuels, la distribution verticale du zooplancton était limitée par la présence d'un monimolimnion anoxique. De même, Swift & Hammer (1979) ont observé que *Daphnia* restait principalement confinée au mixolimnion, sans présenter de déplacement nycthéméral significatif. Néanmoins, la migration verticale nycthémérale du zooplancton reste à ce jour peu étudiée dans les lacs miniers méromictiques, mettant en évidence une lacune importante dans notre compréhension de l'influence des variations de la disponibilité alimentaire et des conditions abiotiques le long de la colonne d'eau sur le comportement de *Daphnia* dans ces écosystèmes uniques.

Par ailleurs, bien que les consommateurs primaires jouent un rôle crucial dans la structuration des réseaux trophiques des lacs méromictiques – en assurant à la fois le transfert d'énergie vers les niveaux trophiques supérieurs et le flux vertical de ressources depuis la chimiocline vers les eaux de surface (Camacho et al., 2001)–, l'étendue de leur niche trophique en fonction de la profondeur demeure totalement inexplorée. Pourtant, l'identification des facteurs déterminants de la largeur de la niche trophique est une question fondamentale en écologie (Fetzer et al., 2015; Schoener, 1974). Traditionnellement mesurée à travers la diversité alimentaire à l'aide d'indices tels que la richesse et l'équitabilité des proies, son évaluation soulève plusieurs défis pratiques, tels que la difficulté de mesurer avec précision l'abondance relative des proies, l'intégration temporelle limitée des données alimentaires, et l'incapacité à prendre en compte les variations dans les taux d'assimilation des proies (Bearhop et al., 2004). Dans ce contexte, l'analyse des isotopes stables constitue une méthode complémentaire largement utilisée pour étudier les niches trophiques et leur structure, car elle permet de mieux comprendre l'origine des éléments et les processus biogéochimiques qui en découlent (Layman et al., 2012; Wada et al., 1991). Le $\delta^{13}$C, avec un fractionnement minimal lors des transferts trophiques, est particulièrement utile pour tracer les sources primaires de carbone dans l'alimentation des consommateurs (Layman et al., 2012; Schilder et al., 2015), tandis que le $\delta^{15}$N est couramment employé pour évaluer les positions trophiques et l'architecture générale des réseaux trophiques (Ek et al., 2015). De précédentes études ont démontré que, dans les lacs méromictiques salins, la matière organique particulaire à la surface présentait la composition isotopique du carbone la plus légère, tandis que le développement de bactéries phototrophes anoxygéniques à l’interface entre les eaux oxiques et sulfidées entraînait un appauvrissement marqué en $^{13}$C et que les valeurs de $\delta^{13}$C deviennaient de nouveau plus légères dans la monimolimnion par rapport à la chimocline (Camacho et al., 2001; Phillips et al., 2021; Pimenov et al., 2008; Savvichev et al., 2014). Cependant, aucune étude à notre connaissance n'a exploré l'influence de ces patterns isotopiques sur les niveaux trophiques supérieurs, notamment pour les organismes filtreurs tel que *Daphnia*. De plus, aucune étude n'a examiné les signatures isotopiques de l'azote dans ces environnements. Étant donné que des variations de température et les apports en nutriments se produisent tout au long de la saison et peuvent affecter les relations trophiques (Ávila et al., 2017), il est également essentiel d’évaluer l’évolution de la largeur de la niche au cours des saisons.

Dans cette étude, nous avons examiné la niche trophique d’une population de *Daphnia pulicaria* vivant dans un lac minier submergé de type méromictique situé à Val-des-Monts, au Québec, Canada. L’objectif général était d’évaluer comment les variations des paramètres abiotiques influencent la niche trophique et les dynamiques des sources de carbone de *D. pulicaria* à travers un gradient de profondeur, en tenant compte des variations entre le jour et la nuit et au cours du début de l’été.

Les objectifs spécifiques de cette étude étaient les suivants :

1. Évaluer comment la diversité, la composition et les signatures isotopiques ($\delta^{13}$C et $\delta^{15}$N) des communautés microbiennes servant de ressources alimentaires à *D. pulicaria* variaient en

réponse aux facteurs abiotiques le long du gradient de profondeur et sur de courtes périodes, aussi bien diurnes qu'au cours du début de l'été ;

2. Examiner la largeur de la niche trophique de *D. pulicaria* en fonction des différences dans les ressources alimentaires disponibles à travers le gradient de profondeur ;
3. Étudier les motifs de migration verticale diurne de *D. pulicaria* en relation avec sa niche trophique.

Afin d'atteindre ces objectifs, nous avons formulé les hypothèses et prédictions suivantes :

1. **H1 :** Les variations abiotiques le long du gradient de profondeur, entre les périodes jour/nuit et au cours du début de l'été influencent significativement la diversité et la composition des communautés microbiennes servant de ressources alimentaires à *D. pulicaria*

   **P1 :** La diversité des communautés microbiennes servant de ressources alimentaires à *D. pulicaria* diminuerait le long du gradient de profondeur, avant de croître à nouveau au fond du monimolimnion, tandis que leur composition serait influencée par des variables corrélées à ce gradient, telles que la température et l'oxygène dissous dans le mixolimnion, ainsi que les concentrations de métaux, d'ammonium et d'ammoniac dans le monimolimnion (Andrei et al., 2015; Čanković et al., 2019; Edberg et al., 2012);
2. **H2 :** Les variations abiotiques le long du gradient de profondeur, entre les périodes jour/nuit et au cours du début de l'été influencent significativement les signatures isotopiques en $^{13}$C et en $^{15}$N de la matière organique particulaire

   **P2 :** La matière organique particulaire contenant les communautés microbiennes serait enrichie en $^{13}$C dans le mixolimnion et dans le monimolimnion, tandis qu'elle serait déplétée à la chimiocline (Phillips et al., 2021; Pimenov et al., 2008; Savvichev et al., 2014)
3. **H3 :** En l'absence de prédateurs visuels et compte tenu des contraintes abiotiques, *D. pulicaria* limite ses déplacements verticaux

   **P3 :** La migration verticale nycthémérale de *D. pulicaria* serait limitée ou absente, puisque d'autres recherches ont montré que de mauvaises conditions alimentaires en profondeur et l'absence de prédation peuvent entraîner une migration réduite ou absente (Beklioglu et al., 2008; Dini & Carpenter, 1992)

   **P4 :** Ainsi, *D. pulicaria* afficherait des signatures isotopiques spécifiques à la profondeur, avec un signal enrichi en $\delta^{13}$C dérivé du phytoplancton abondant en surface, et un signal appauvri en $\delta^{13}$C dérivé des sources microbiennes en profondeur, reflétant des sources de carbone primaires distinctes.

En nous concentrant sur *D. pulicaria*, un consommateur primaire clé, nous apportons de nouvelles perspectives sur les dynamiques écologiques dans les lacs miniers méromictiques, où des conditions hostiles comme l'anoxie et la faible disponibilité alimentaire défient la vie des zooplanctons. À notre connaissance, cette étude est la première à évaluer *in situ* l'influence des paramètres abiotiques sur la niche trophique et la distribution verticale de *D. pulicaria* dans un tel écosystème, contribuant ainsi à une meilleure compréhension de la dynamique des réseaux trophiques dans les lacs miniers et offrant des perspectives sur les processus écologiques au sein des sites miniers abandonnés.

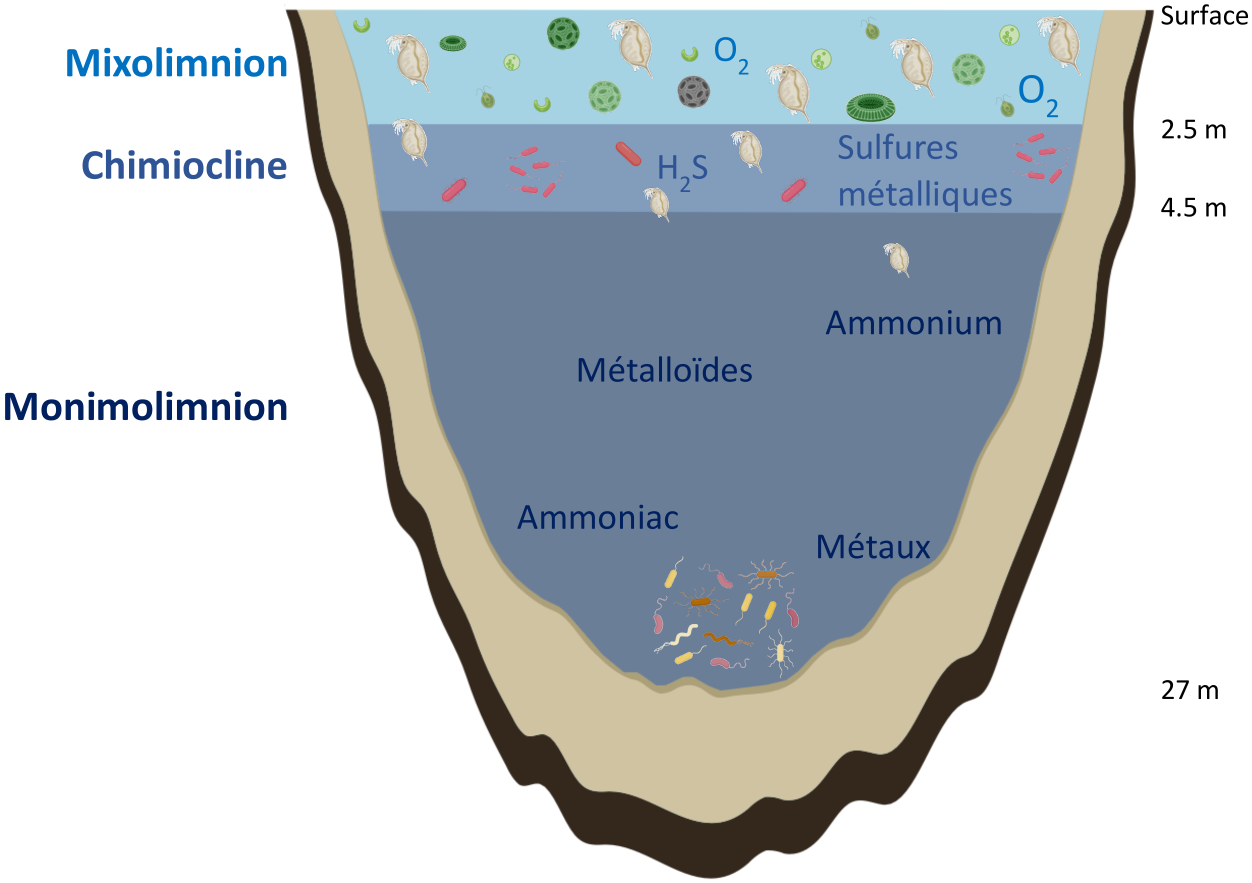

Figure : Structure vertical des compartiments abiotiques et écologiques d'un lac méromictique.

Les résultats de ce projet de recherche seront maintenant présentés sous forme d'un article qui sera bientôt soumis à Hydrobiologia pour être revu par les pairs.

# CHAPITRE 1

# Basal dietary resources and dietary niche of the keystone grazer, Daphnia pulicaria, in an abandoned, meromictic mining lake

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## Abstract

Our study examined how diel, intra-seasonal, and depth-related abiotic variations influence basal microbial communities and the dietary niche of Daphnia pulicaria in a meromictic mining pit lake in Val-des-Monts, Quebec. We hypothesized that depth, time of day, and seasonal changes would affect microbial community composition and the $\delta^{13}$C and $\delta^{15}$N isotopic signatures of particulate organic matter (POM), which serve as dietary resources for D. pulicaria. We also expected D. pulicaria to exhibit depth-specific isotopic signatures and limited vertical migration due to anoxic deep layers and the absence of visual predators such as fish. Using 16S/18S rRNA gene sequencing, we found that while *D. pulicaria*'s microbial food resource diversity remained stable across depths, there were depth-related shifts in microbial community composition and $\delta^{13}$C and $\delta^{15}$N signatures of POM. These depth-related shifts indicated a transition from photosynthetically derived carbon at the surface to deeper carbon sources, likely influenced by the dominance of green sulfur bacteria and methanotrophic activity at the chemocline, and chemosynthetic use of respired $CO_2$. *D. pulicaria*'s C-isotopic dietary niche variation was wider in the mixolimnion than in the monimolimnion, possibly reflecting a higher availability of dietary resources in this oxygenated layer. As expected, *D. pulicaria* did not exhibit diel vertical migration, likely due to anoxic conditions in the monimolimnion and the absence of fish predation. These findings provide insight into food-web structure in meromictic pit lakes and their implications for biogeochemical cycling and ecosystem resilience.

Keywords

18S/16S rRNA, $\delta^{13}C$, $\delta^{15}N$, mining pit lake, *Daphnia*, microbial communities

Statements and Declarations

Competing interests : The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## 1.1 Introduction

Globally, the closure of open-pit mines has resulted in the formation of thousands of artificial lakes, representing a growing component of inland aquatic ecosystems (Kemanga et al., 2024). Yet, the ecology of meromictic mining pit lakes are generally understudied (Gulati et al., 2017). Indeed, when dewatering operations end as a mine closes, open-pit mines gradually fill with groundwater and rainfall, creating pit lakes (Lund & Blanchette, 2023; Wu et al., 2004). As the lake forms and water accumulates in contact with exposed rock surfaces, it interacts with primary and secondary minerals under oxidizing conditions, leading to mineral dissolution that alters water quality (Atanacković et al., 2013). These lakes often exhibit high depth-to-surface-area ratios, flat bottoms, and steep sides, which inhibit wind-driven mixing of surface and deep waters, increasing the likelihood of meromixis compared to natural lakes (Blanchette & Lund, 2016; Boehrer & Schultze, 2006). This stratification results in an oxygenated mixolimnion at the surface and an anoxic, turbid monimolimnion at the deepest layers, separated by a chemocline rich in metal sulfides (Boehrer & Schultze, 2008; Gulati et al., 2017). These unique conditions promote the accumulation of metals and metalloids at the lake bottom while limiting light penetration and the availability of organic matter in these deeper waters (Goździejewska et al., 2019; von Gunten et al., 2019). Microorganisms, which were and are present on mine rocks, soils, and wood, and in groundwater and surface waters must and have adapted to these artificial aquatic ecosystems (Lhoste et al., 2023).

Microbial research in meromictic lakes remains fragmented – focusing on either prokaryotes or eukaryotes, and rarely incorporating short-term temporal or diel (day-night) variability. Previous studies on microbial communities revealed distinct vertical patterns in the composition and structure of prokaryotic and eukaryotic assemblages, shaped by depth-related environmental gradients such as oxygen concentration, light availability, and metal concentrations. In a mountain meromictic lake, Oikonomou et al. (2014) demonstrated that mixotrophic protists played a key ecological role through substantial grazing activity in the oxic mixolimnion, whereas grazing pressure at the chemocline had limited influence on prokaryotic abundance and was negligible in the anoxic layers. A follow-up study by the same authors (Oikonomou et al., 2015) found that protist communities in the mixolimnion underwent the greatest seasonal shifts, diversity peaked at the suboxic chemocline in spring and summer, and monimolimnion assemblages remained the most stable throughout the year. In a meromictic sulfur mine lake, Wilk-Woźniak & Żurek (2006) reported that the phytoplankton community comprised a small number of species – including cyanoprokaryotes, chrysophytes, diatoms, euglenophytes, dinophytes, cryptophytes, and chlorophytes – with diatoms present even in the anoxic monimolimnion. Krasnova et al. (2015) further noted that between the aerobic and anoxic water layers, green and purple sulfur bacteria, alongside eukaryotic biota, included mixotrophic organisms and species tolerant to anoxia. Moreover, Edberg et al., (2012) showed that in a former uranium open-pit mine, a shift from oxic conditions in the mixolimnion to anoxic, alkaline, and metal- and sulfide-rich conditions in the monimolimnion coincided with a marked change in the bacterial community. While the mixolimnion resembled other temperate freshwater systems, the monimolimnion exhibited higher bacterial diversity, dominated by phototrophic green sulfur bacteria and ferric iron and sulfate reducers. Andrei et al. (2015) also observed that bacterial diversity declined from the surface through the chemocline but increased again in deeper water strata in a hypersaline meromictic lake. Collectively, these studies highlight how vertical environmental gradients shape microbial community composition and diversity in meromictic lakes, ultimately influencing the structure of basal food resources available to higher organisms, including zooplankton such as *Daphnia pulicaria*. However, Moreover, studies with integrative approaches that consider multiple microbial domains across fine spatial and temporal scales remain limited.

The extent to which these depth-related microbial patterns in mercomictic lakes influence higher trophic levels organisms, such as zooplankton, remains poorly understood. Numerous studies have shown that, under conditions where meromixis does not occur and the water column mixes completely, the primary driver of *Daphnia*'s diel vertical migration in lake is the presence of fish (Ringelberg, 1991; Zaret & Suffern, 1976). In a meromictic lake where fish were present, Boronat & Miracle (1997) reported that *Daphnia* exhibited a small migration amplitude, reflecting a trade-off between avoiding predators and maximizing food intake. In contrast, other research indicates that poor food conditions in deeper water layers and fishless environments can lead to reduced or absent migration (Beklioglu et al., 2008; Dini & Carpenter, 1992), suggesting that food availability may become the dominant driver under such conditions in meromictic pit lakes. Indeed, Zadereev & Tolomeyev (2007) reported that, in the absence of visually guided predators, zooplankton vertical distribution was restricted to the oxic layers of the water column. Similarly, Swift & Hammer (1979) observed that *Daphnia* remained confined to the mixolimnion without significant diel movement. Nonetheless, diel vertical migration in *Daphnia* remains unstudied in meromictic pit lakes, highlighting a critical gap in our understanding of how food availability and abiotic conditions influence its behavior in these unique ecosystems.

Additionally, while primary consumers play a crucial role in shaping the food-web structure in meromictic lakes - facilitating both the transfer of energy to higher trophic levels and the vertical flux of resources from the chemocline to surface waters (Camacho et al., 2001) - the variation of their dietary niche across depths remains entirely unexplored. In this context, stable isotope analysis is a widely used technique for examining food webs and their structure, as it provides valuable insights into the origin of elements and subsequent biogeochemical processes (Layman et al., 2012; Wada et al., 1991). $\delta^{13}C$, with minimal fractionation during trophic transfers, is particularly useful for tracing primary carbon sources in consumers' diets (Layman et al., 2012; Schilder et al., 2015), while $\delta^{15}N$ is commonly employed to assess trophic positions and the overall architecture of food webs (Ek et al., 2015). Previous studies showed that, in saline meromictic lakes, particulate organic matter at the surface had the lightest carbon isotopic composition, while the development of anoxygenic phototrophic bacteria at the interface between oxic and sulfidic waters led to a marked depletion in $^{13}$C, and $\delta^{13}$C values became lighter again in the monimolimnion compared to the chemocline (Camacho et al., 2001; Phillips et al., 2021; Pimenov et al., 2008; Savvichev et al., 2014). However, to our knowledge, no study has investigated the influence of these isotopic patterns on higher trophic levels in meromictic pit lakes, particularly for filter-feeding organisms such as *Daphnia*. In addition, nitrogen isotopic signatures have not been examined in these environments. Since variations in temperature and nutrient inputs occur throughout the season and can lead to changes in trophic networks (Ávila et al., 2017), it is also important to assess dietary niche over the course of seasons.

We investigated the basal microbial communities acting as dietary resource and the dietary niche of a *Daphnia pulicaria* population across depth, time, and abiotic factors in a meromictic mining pit lake. The objectives were: 1) To assess how the diversity, community composition, and isotopic signatures ($\delta^{13}$C and $\delta^{15}$N) of microbial communities serving as dietary resources for *Daphnia pulicaria* varied in response to abiotic factors along the depth gradient; 2) To examine the isotopic dietary niche of *D. pulicaria* in relation to differences in available dietary resources across the depth gradient. the diversity of microbial communities would decrease along the depth gradient and increase again in the lower monimolimnion, while community composition would be influenced by variables correlated with this gradient, such as temperature and dissolved oxygen in the mixolimnion, as well as metal, ammonium, and ammonia concentrations in the monimolimnion (Andrei et al., 2015; Čanković et al., 2019; Edberg et al., 2012). We predicted that particulate organic matter (POM) containing microbial communities would be enriched in $^{13}$C in both the mixolimnion and the monimolimnion, while being depleted at the chemocline (Phillips et al., 2021; Pimenov et al., 2008; Savvichev et al., 2014). We expected that *D. pulicaria* would exhibit depth-specific isotopic signatures consistent with the POM, with an enriched $\delta^{13}$C signal derived from phytoplankton abundant at the surface, and a depleted $\delta^{13}$C signal derived from microbial sources at greater depths, reflecting distinct primary carbon sources. Our study uniquely offers new insights into the ecological dynamics of meromictic mining lakes, where anoxia, elevated metal concentrations, and limited food availability pose major challenges to aquatic life from microbial communities to zooplanktonic life.

## 1.2 Materials and methods

We characterized microbial resource communities using high-throughput sequencing, targeting prokaryotes and eukaryotes. We applied stable isotope analyses, based on $\delta^{13}$C and $\delta^{15}$N ratios, to the particulate organic matter (POM) and *D. pulicaria* to infer dietary niche of the *Daphnia* in relation to the POM. Diurnal vertical migration of the *Daphnia* was investigated to understand if their foraging varied with lake depth despite lack of fish in the lake. Diel changes in microbial communities were also examined to assess potential variations in basal food resources between day and night.

### 1.2.1 Site description and field sample collection

Due to its favorable geology, Quebec (Canada) has contributed and contributes significantly to the mining industry, by extraction of resources such as gold, iron, copper, and rare earth elements (Marshall, 2021). However, Canada's long history of unregulated mining practices has led to numerous abandoned sites being left without environmental restoration once mining activities ceased (Hogan & Tremblay, 2006). Although regulations on mine closure and restoration were established in 1991, as of 2024 Quebec still harbors 346 abandoned and flooded mine sites requiring varying degrees of rehabilitation (MRNF, 2024).

Our study lake (45°38'19"N, 75°36'31"W) is located within the Blackburn Mine (MB), which is an old mica mine located 5 km northeast of the village of Perkins in the municipality of Val-Des-Monts, western Quebec (Canada). Mining began at this site in the early 1870s with phosphate extraction from apatite, but as demand declined in the late 1880s, production shifted to mica. Operations continued until 1942, when the site was abandoned without any environmental restoration. Today, the mine is completely flooded. The site produces contaminated neutral drainage, leading to the formation of a meromictic mining lake with a pH close to neutrality. The only zooplankton species inhabiting this environment is *Daphnia pulicaria*, with *Chaoborus* spp. larvae being the only known observed predators. The lake is fishless.

Sampling depths were determined based on vertical profiles of temperature and dissolved oxygen, which were measured using multiparameter probes (RBR Maestro3, RBR Ltd®, Ottawa, ON, Canada). Water and zooplankton samples were collected during both daytime (around solar noon, i.e. around 1 p.m.) and nighttime (before the start of sunset, i.e., prior to 5 a.m.) on three sampling campaigns between May and June 2024, with a 20 day interval between each sampling event (May 17, June 6 and 26). The lunar phases during these dates were Waxing Gibbous, New Moon, and Waning Gibbous, respectively. Water was collected using a horizontal water bottle (Wildco®, Yulee, FL, USA) at the surface, and at depths of 3, 6, and 18 meters, corresponding to the mixolimnion, chemocline, and upper and deeper parts of the monimolimnion, respectively. For each depth, water samples were stored in three 1 L polyethylene bottles (Nalgene®, Rochester, NY, USA), which had been previously acid-washed with 10% HCl and sterilized, for isotopic, taxonomic, and abiotic parameters analysis. *D. pulicaria* were collected using a Wisconsin closing zooplankton net (50 cm diameter, 2 meters long, 54 µm mesh) in the mixolimnion (0–2 m), chemocline (2.5–4.5 m), and monimolimnion (5–7 m), then stored in acid leached (10% HCl) plastic cups. On the last sampling day, only water samples could be collected as the daphnids were absent, despite numerous attempts to find and collect them. All samples were stored at 4°C in the dark during transport. Water samples for microbial analyses and particulate organic matter (POM) isotopic analyses were filtered through a combusted (525°C for 4h) glass fiber filter (0.7 µm nominal pore size, Whatman™ glass microfiber filters, Grade GF/F, Cytiva®, Marlborough, MA, USA) on the day of sampling.

### 1.2.2 Environmental parameter analyses

Temperature and dissolved oxygen (DO) were measured in situ along the water column during sampling using a HOBO® Dissolved Oxygen Logger (U26-001, Onset Computer Corporation, Bourne, MA, USA). Water samples were filtered immediately after collection in the field through 0.45 µm polyethersulfone filters (Sarstedt®, Numbrecht, Germany) for analyses of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), nitrite ($NO_2^-$), and nitrate ($NO_3^-$). DOC and DIC were measured using an OI Analytical Aurora 1030W TOC Analyzer (College Station, TX, USA) with persulfate oxidation. $NO_2^-$ and $NO_3^-$ concentrations were determined with an OI Analytical Flow Solution® 3100 continuous flow analyzer (College Station, TX, USA) using alkaline persulfate digestion coupled with a cadmium reactor, following standard protocols (Patton & Kryskalla, 2003). For ammonium and ammonia ($NH_x$) analysis, water samples were filtered through 0.20 µm polyethersulfone filters (Sarstedt®, Numbrecht, Germany) and measured with the Flow Solution® 3100 autosampler (College Station, TX, USA), using EPA Method 350.1 based on the chloramine reaction with salicylate to form indophenol blue dye. All analyses were conducted at the analytical laboratory of the Interuniversity Research Group in Limnology, at the University of Quebec in Montreal (GRIL-UQAM).

Water samples collected during the day only (one time point per sampling campaign) were also filtered through 0.20 µm polyethersulfone filters (Sarstedt®, Numbrecht, Germany) to measure trace metal elements. The filtrate was transferred to 15 mL metal-free tubes, acidified with 2% nitric acid, and analyzed using an Agilent 8900 ICP-QQQ (Agilent Technologies, Inc., Santa Clara, CA, USA) at the UQAM-Environmental Analysis Laboratory of the Institute of Environmental Sciences (LAE-ISE). To avoid issues of co-correlation among metals, we retained copper (increasing with depth) and iron (decreasing with depth) as representative elements, in addition to the sum of rare earth elements.

### 1.2.3 *Daphnia* characteristics

Counting and body length measurements of the daphnids were performed on 50 mL subsamples using a high-resolution Olympus® stereomicroscope (Hachioji, Tokyo, Japan) at 20x magnification. *D. pulicaria* density was calculated as number of individuals per liter, assuming a 100% net filtration efficiency.

### 1.2.4 Microbial gene amplification and sequencing

DNA of microorganisms in the POM was extracted from the previously obtained filters using the DNeasy PowerWater kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The Eukaryotic V5 region of the 18S rRNA was amplified using the primer pair E960F (5′-GGCTTAATTTGACTCAA- CRCG-3′) (Van De Peer et al., 2000) and NSR1438R (5′-GGGCATCACAGACCTGTTAT-3′) (Gast et al., 2004). The bacterial V3–V4 region of the 16S rRNA gene was amplified using the primer pair B341F (5′-CCTACGGGAGGCAGCAG- 3′) and B785R (5′-GACTACHVGGGTATCTAATCC-3′) (Klindworth et al., 2013). PCR reactions were performed using the Phusion Hot Start II polymerase (Thermo Scientific™, Thermo Fisher Scientific Inc., Waltham, MA, USA), following the manufacturer's instructions (Supplementary Information (SI) Table S1 ). Sequencing was carried out for both domains with the Illumina Miseq using MiSeq Reagent Kit v.3 (600 cycles, illumina) at the CERMO-FC genomic platform (Centre excellence en recherche sur les maladies orphelines - Fondation Courtois). A negative control for the DNA extraction kit was prepared by filtering sterile Milli-Q® water through a combusted (525°C for 4h) glass fiber filter (0.7 µm nominal pore size, Whatman™ glass microfiber filters, Grade GF/F, Cytiva®, Marlborough, MA, USA), following the same protocol as for environmental samples. A PCR negative control was also included, and all controls were sequenced for both domains. The raw reads were deposited into the National Center for Biotechnology Information (NCBI) under the BioProject ID 1268407.

### 1.2.5 Stable isotope analyses

POM sample preparation followed the method described by McCallister & Del Giorgio (2008). Filters for stable isotope analyses of POM were dried at 45°C, acid fumed overnight with HCl and then dried again at 45°C. *D. pulicaria* individuals were pooled by sample while still alive, then rinsed and depurated for 24 hours in deionized water to void their gut contents prior to isotopic analysis. Only individuals that survived the depuration in deionized water were retained for further processing. They were subsequently acidified with 10% HCl and dried overnight at 45°C. For each sample, a minimum of 4.44 mg of *D. pulicaria* dry matter was used for the isotopic analyses. Samples were weighted in smooth-walled tin capsules to obtain the same amount of $CO_2$ and $N_2$ for all samples and reference materials. Stable carbon and nitrogen isotopes were measured at the UQAM stable isotope's laboratory of the Research Centre in Earth System Dynamics (GEOTOP), using a Micromass® model IsoPrime 100 isotope ratio mass spectrometer (Elementar Americas Ltd., Ronkonkoma, NY, USA) coupled to an Elementar® Vario MicroCube elemental analyser in continuous flow mode (Elementar Americas Ltd., Ronkonkoma, NY, USA). For carbon analysis, two internal reference materials ($\delta^{13}C$= -28.74 ± 0.02 ‰ and -11.80 ± 0.03‰) were used to normalize the results on the NBS19-LSVEC scale, and a third reference material ($\delta^{13}C$= -17.06 ± 0.02 ‰) was analyzed as an unknown to assess the exactness of the normalization. For nitrogen analysis, two internal reference materials ($\delta^{15}N$ = -0.06 ± 0.09 ‰ and +14.81 ± 0.08 ‰) were used to normalize the results on the AIR scale (IAEA-N1, IAEA-N2, & IAEA-N3), with a third reference material ($\delta^{15}N$ = 6.35 ± 0.13 ‰) analyzed as an unknown to assess the exactness of the normalization. Results are given in delta units (δ) in ‰ vs VPDB for carbon (Vienna Pee Dee Belemnite standard) and ‰ vs AIR for nitrogen (atmospheric $N_2$ standard). The overall analytical uncertainty ($\sigma 1$) is better than ± 0.1‰ for carbon and ± 0.2‰ for nitrogen. This uncertainty is based on the propagation of uncertainties from the normalization of the internal reference materials and the samples but does not account for the homogeneity or representativity of the sample.

### 1.2.6 Bio-informatic analyses

To identify amplicon sequence variants (ASVs), 16S/18S rRNA gene sequences were filtered, processed, and analyzed using a modified DADA2 pipeline (v.1.26.0, Callahan et al., 2016) in R v4.2.2, to manage the quality of forward and reverse reads. For bacterial sequences, forwards and reverse reads were truncated at positions 280 and 240, respectively. For eukaryote sequences, forwards and reverse reads were truncated at positions 260 and 230, respectively. Microbial taxonomy was assigned with the DADA2 package using the assign taxonomy function and the SILVA SSU database (v.138.1) for bacterial sequences (Quast et al., 2013) and the PR2 SSU database (v.5.0.0) for eukaryote sequences (Guillou et al., 2013). ASVs in negative controls (extraction kits and PCR) were removed from all output sequences, with the decontam R package (v.1.18.0, Davis et al., 2018) using the is Contaminant function. Then, bacterial and eukaryotes community tables were rarified to 1823 and 2459 ASVs, respectively. For the eukaryotic dataset, samples with insufficient sequencing depth were excluded, namely D3-H2 (day 3, hour 2) in the upper monimolimnion (6 m) and D2-H2 and D3-H2 in the deeper monimolimnion (18 m). In addition, sequences affiliated with vertebrate eukaryotes taxa were removed to retain only microbial eukaryotes.

### 1.2.7 Statistical analyses

Statistical analyses were performed with Rstudio Server 2024.04.24 (v.4.4.0). Data normality was assessed using the Shapiro-Wilk test, and since the data did not meet the assumptions of normality, non-parametric statistical tests were used. For all test, differences were considered statistically significant at $p$-value $< 0.05$.

Relationships between abiotic parameters and depth were determined through Spearman correlation analyses using the ggplot2 package (v.3.5.1) and the cor.test function. To compare abiotic parameters between sampling days across depths, the non-parametric Kruskal-Wallis test was applied, using the compare_means function from the ggpubr package (v.0.6.0), as more than two groups (three sampling days) were compared. Using the same package, wilcoxon tests were used to examine differences in abiotic parameters between diel periods (day or night) across depths and sampling days, as only two sampling groups were compared. Due to the absence of replicates within one sampling day, no statistical test could be applied to assess day-to-day or diel variation in metal concentrations. To assess differences in *D. pulicaria* body length and density between depths, the same Kruskal-Wallis and Wilcoxon tests were applied to compare values between sampling days and diel periods across depths.

To compare the bacterial and eukaryotes communities across the depth gradient, between days, and between diel period (day or night), a series of diversity indices related to taxonomy were used, including the Shannon index, the observed richness, and the evenness, utilizing the Phyloseq package (v.1.48.0, McMurdie & Holmes, 2013). The same Wilcoxon and Kruskal-Wallis tests as described above were applied, followed by a post-hoc Dunn test for multiple pairwise comparisons when the Kruskal-Wallis test was significant. Relationships between abiotic parameters and α-diversity indices were determined through Spearman correlation analyses using the ggplot2 package (v.3.5.1) and the cor.test function.

To visualize dissimilarities in bacterial or eukaryotes community structures (β-diversity) across the depth gradient, a Principal Coordinates Analysis (PCoA) was performed on the rarefied relative abundance data using a Bray-Curtis dissimilarity matrix with the Vegan package and the vegdist function (v.2.6.8, Oksanen et al., 2025). To assess the influence of depth, sampling day, and diel period on bacterial or eukaryotic community composition, a permutational multivariate analysis of variance (PERMANOVA), using the Vegan package (v.2.6.8, Oksanen et al., 2025) and the adonis2 function, with significance tested using the default 999 permutations. To examine the relationships between bacterial or eukaryotic community composition and temperature, dissolved oxygen, DOC, DIC, $NO_3^-$, and $NH_x$, a distance-based redundancy analysis (db-RDA) was performed on Hellinger-transformed community data, followed by a sequential contrast analysis of variance (ANOVA) with one degree of freedom to test specific effects. Variance partitioning was performed using the varpart function from the Vegan package (v.2.6.8; Oksanen et al., 2025) on the significant variables identified by db-RDA to quantify their contributions to microbial community composition. Additionally, a db-RDA analysis (followed by an ANOVA) was performed including metal concentrations (Cu, Fe, and ΣREEs), but this analysis was restricted to samples collected during the day and conducted only for the bacterial domain, as the eukaryotic dataset was too limited due to sample loss after rarefaction.

Differences in $\delta^{13}C$ and $\delta^{15}N$ values across the depth gradient were evaluated using the Kruskal-Wallis test, followed by Dunn's post-hoc test with Bonferroni correction for multiple comparisons when significant results were found. To compare $\delta^{13}C$ and $\delta^{15}N$ values between diel periods across depths, Wilcoxon tests were applied, while differences between sampling days were tested using the Kruskal-Wallis test. In addition, Pearson correlation analyses were conducted to assess the influence of mean body length, density, abiotic parameters, and depth on isotope values ($\delta^{13}C$ and $\delta^{15}N$).

## 1.3 Results

### 1.3.1 Abiotic parameters along the depth gradient in the mining pit lake

Table 1. Mean ± standard deviation of environmental variables measured at each depth (0, 3, 6, and 18 meters) in the open-pit mining lake, averaged across all sampling days and diel periods (day/night). For temperature, dissolved oxygen (DO), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), nitrate ($NO_3^-$), and ammonia + ammonium ($NH_x$), n = 6; for copper (Cu), iron (Fe), and sum of rare earth elements (ΣREEs), n = 3.

| **Depth (m)** | **Temperature (°C)** | **DO (mg/L)** | **DIC (mg/L)** | **DOC (mg/L)** | **$NO_2^-$ (mg/L)** | **$NO_3^-$ (mg/L)** | **$NH_x$ (mg/L)** | **Cu (nmol/L)** | **Fe (nmol/L)** | **ΣREEs (nmol/L)** |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 20,18 ± 3.53 | 8.88 ± 0.80 | 31.71 ± 3.11 | 5.48 ± 0.73 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.03 ± 0.02 | 17.12 ± 0.73 | 533.61 ± 500.16 | 0.89 ± 0.13 |
| 3 | 15.11 ± 0.68 | 5.05 ± 1.04 | 46.15 ± 5.38 | 5.12 ± 0.23 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.04 ± 0.04 | 13.61 ± 11.03 | 436.73 ± 139.97 | 1.59 ± 0.36 |
| 6 | 11.24 ± 0.12 | -0.02 ± 0.19 | 58.70 ± 8.31 | 4.50 ± 0.52 | 0.00 ± 0.00 | 0.00 ± 0.01 | 0.76 ± 0.15 | 1.20 ± 0.41 | 1941.62 ± 400.28 | 3.51 ± 0.68 |
| 18 | 8.90 ± 0.10 | -0.21 ± 0.01 | 60.10 ± 6.45 | 4.30 ± 0.42 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.10 ± 0.04 | 0.65 ± 0.28 | 1262.70 ± 49.48 | 4.20 ± 0.09 |

Along the water column, we observed a marked thermal and chemical stratification (Table 1 ; and SI Table S2). Surface waters were warm (20.2 ± 3.5 °C) and well oxygenated (8.9 ± 0.8 mg/L), whereas temperature declined progressively with depth, reaching 8.9 ± 0.1 °C at 18 m. Dissolved oxygen concentrations also decreased with depth and were undetectable at both 6 m (–0.02 ± 0.2 mg/L) and 18 m (–0.21 ± 0.01 mg/L), indicating anoxic conditions. DIC concentrations decreased with depth, from 31.7 ± 3.1 mg/L at the surface to 4.3 ± 0.4 mg/L at 18 m, while DOC increased from 5.5 ± 0.7 mg/L to 6.5 ± 0.4 mg/L. $NH_x$ increased with depth, from 0.03 ± 0.02 mg/L at the surface to 1.10 ± 0.04 mg/L at 18 m. $NO_2^-$ was found only in a single sample (collected on the second sampling day, during the day, at 6 m depth at a concentration = 0.01 mg/L; SI Table S2), whereas $NO_3^-$ was only detected at the surface (0.01 ± 0.01 mg/L) and was below detection limits at all other depths. Trace metal concentrations also varied along the vertical gradient, with dissolved iron (Fe) increasing from 533.6 ± 500.2 nmol/L at the surface to a peak of 1941.6 ± 400.3 nmol/L at 6 m, before slightly declining at 18 m (1262.7 ± 49.5 nmol/L). Copper (Cu) concentrations dropped steeply with depth, from 17.1 ± 0.7 nmol/L at the surface to 0.65 ± 0.28 nmol/L at 18 m. Finally, the sum of rare earth elements (ΣREEs) also increased with depth, from 0.89 ± 0.13 nmol/L at the surface to 4.20 ± 0.09 nmol/L at 18 m.

Therefore, we established that during early summer 2024, the mixolimnion extended from the surface to approximately 2.5 m and was characterized by elevated dissolved oxygen concentrations and relatively high-water transparency. At around 2 m, a thermocline was present, marked by a rapid decrease in temperature with depth. Below this, between ~2.5 and 4.5 m (depending on water level fluctuations), a chemocline was present, marked by hydrogen sulfide accumulation and reduced visibility. The monimolimnion, extending from the chemocline to a depth of 27 m at the bottom of the open-pit mine, was characterized by anoxic conditions, high concentrations of suspended particulates, and zero visibility.

All measured abiotic parameters, except $NO_2^-$, were significantly correlated with depth (SI Table S3). Temperature, dissolved oxygen, DOC, and $NO_3^-$ were negatively correlated with depth. In contrast, DIC and $NH_x$ had positive correlations with depth. Among metals, copper was negatively correlated with depth, whereas ΣREEs and Fe were positively correlated.

No significant differences in temperature, dissolved oxygen, DOC, DIC, $NO_3^-$, and $NH_x$ were observed between sampling days at any depth (SI Table S4), nor between diel period across all depths (SI Table S5).

### 1.3.2 Basal microbial communities taxonomy along the depth gradient

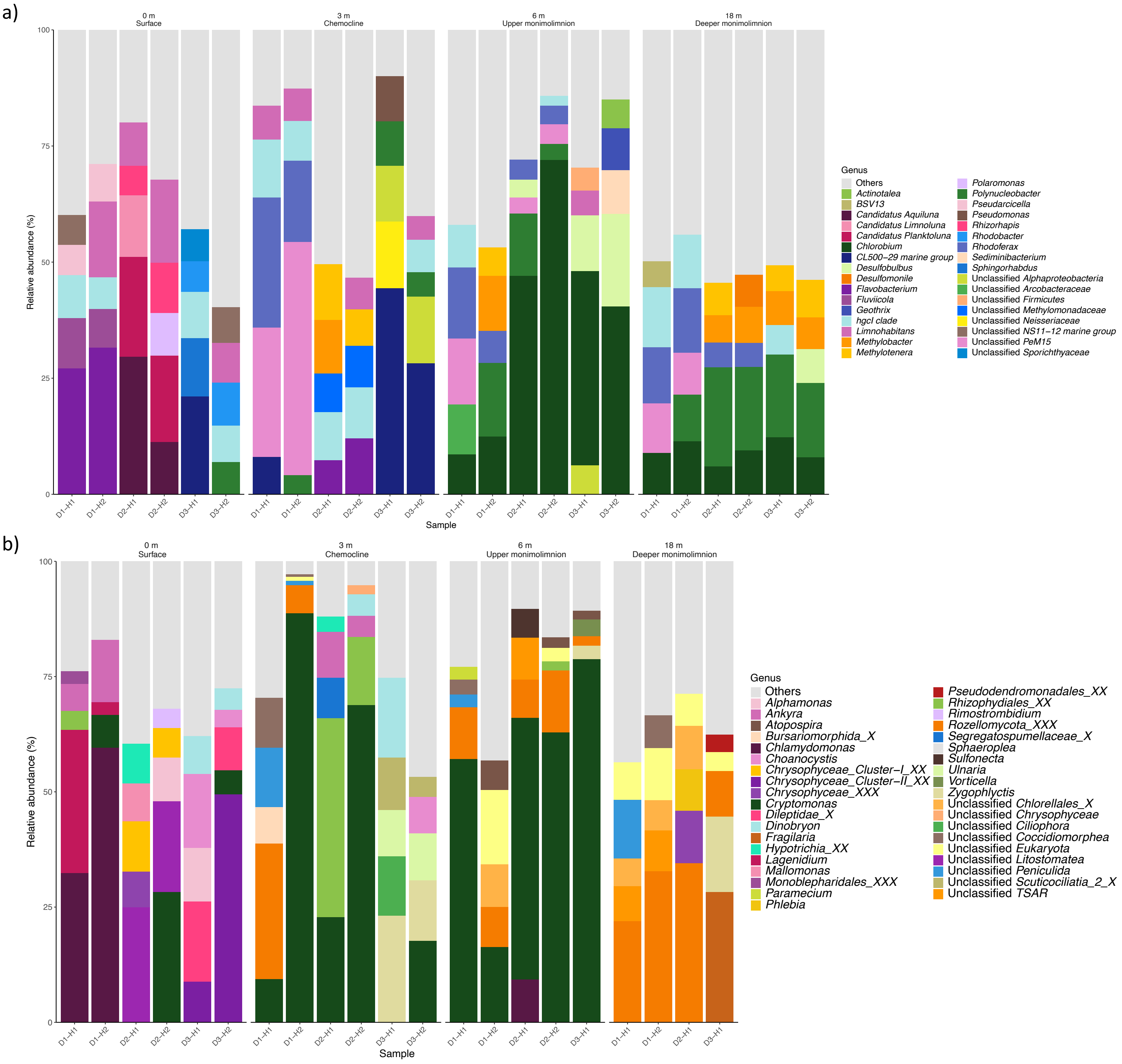

Figure 1. Relative abundance (% of total reads) of bacterial (a) and eukaryote (b) taxa at the genus level, based on 16S and 18S rRNA gene sequencing. D, day; H, hour (H1: Night, H2: Day).

For the bacterial domain, the predominant phyla at the surface and 3 m were *Proteobacteria*, *Actinobacteriota*, and *Bacteroidota*, while at 6 and 18 m, these three phyla remained dominant, but *Desulfobacterota*, absent from surface layers, also became abundant (SI Figure S1a). Taxa such as *Campylobacterota* and *Firmicutes* were present at 6 and 18 m but were absent at the surface and 3 m. At the genus level, the most abundant taxa at the same depth varied between sampling days, particularly at the surface and 3 m depths (Figure 1a). For instance, at the surface, *Flavobacterium* dominated on Day 1 (D1), whereas *candidatus (cand.) Aquiluna* and *cand. Planktoluna* were more prevalent on Day 2 (D2), and *CL500-29 marine group* on Day 3 (D3). Similarly, at 3 m, unclassified (unc.) *PeM15* and *Rhodoferax* were dominant on D1, while *CL500-29 marine group* and unc. *Alphaproteobacteria* were most common on D3. Despite these variations across days, the most abundant taxa remained relatively consistent between diel periods within the same sampling day. At 6 m, *Chlorobium* dominated nearly all samples, while at 18 m, *Polynucleobacter* dominated most samples. In addition, methanotroph taxa such as *Methylobacter*, *Methylotenera*, unc. *Methylomonadaceae*, and unc. *Methylophilaceae* were found in all samples at 18 m (SI Figure S2).

More eukaryotic genera were identified compared to bacterial genera, but the eukaryotic domain exhibited greater variability between sampling days and also differed between diel periods, especially at the surface and 3 m. At the division level, *Cryptophyta* was the most abundant at 6 m, whereas *Opisthokonta* dominated at 18 m (SI Figure S1b). At the genus level, *Cryptomonas* was present in almost all samples at 3 m and was the most abundant taxon at 6 m (Figure 1b). *Rozellomycota_XXX* was the only taxa consistently present across all four samples at 6 and 18 m.

### 1.3.3 Alpha diversity of microbial communities and correlation with abiotic parameters

Alpha diversity indices of bacterial and eukaryotic communities were measured with the Shannon index (SI Figure S3), observed richness (SI Figure S4), and Pielou’s evenness (SI Figure S5). The bacterial Shannon index decreased steadily along the water column until 6 m, before increasing again at 18 m, and showed a significant correlation with depth (SI Table S6). However, significant differences in Shannon diversity indices were observed only between the surface and 6 m (SI Table S7). Additionally, Pielou’s evenness and observed richness in bacterial communities showed no significant variation across depths. For the Eukaryotic domain, alpha diversity indices did not vary significantly with depth (SI Table S6).

### 1.3.4 Beta diversity of microbial communities and correlation with abiotic parameters

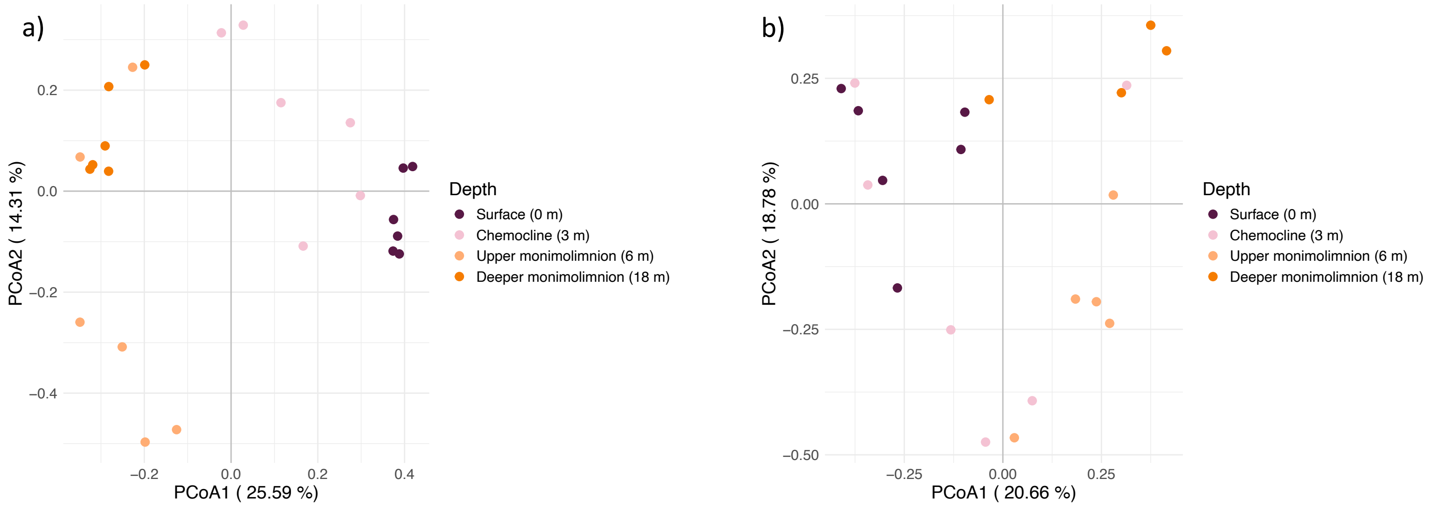

Figure 2. Principal coordinates analysis (PCoA) ordination of bacterial (a) and eukaryote (b) community variance based on a Bray-Curtis dissimilarity matrix.

A principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarities showed a separation between communities at 0 and 3 m and at 6 and 18 m along the first axis (PCoA1), which explained 25.59% and 20.66% of the total community variation in the bacterial and eukaryotic domains, respectively (Figures 2a and 2b). While bacterial communities at 0 m and 18 m each formed distinct clusters, communities at 3 m and 6 m were more dispersed. In contrast, eukaryotic communities were more dispersed overall. The PERMANOVA analysis revealed that depth significantly influenced both bacterial and eukaryotic community composition, explaining 18.6 and 13.6 % of the variance for each community, respectively (SI Table S8). Sampling day also had a significant effect on bacterial and eukaryotic community composition explaining 10 and 12.4 % of the variance for each community, contrary to diel period which did not. Interaction effects between depth, sampling day, and diel period were also not significant for either domain.

The ANOVA by contrast on the Bray-Curtis dissimilarity index (SI Table S9) revealed that temperature significantly influenced both bacterial and eukaryotic community composition. Dissolved oxygen was also correlated with both communities. For DOC and DIC, only the eukaryotic communities were significantly influenced, while bacterial communities showed no significant response. Among nutrients, $NH_x$ had a significant effect on bacterial domain, whereas $NO_3^-$ did not significantly impact bacterial or eukaryotic communities. The db-RDA output graphs (Figures 3a and 3b) showed that surface (0 m) communities for both domains were positively associated with higher temperature and dissolved oxygen levels. In addition, eukaryotic communities at 0 and 3 m exhibited a positive correlation with DOC, which is more abundant near the surface. At 6 and 18 m, where $NH_x$ concentrations were highest, bacterial communities were positively associated with this variable.

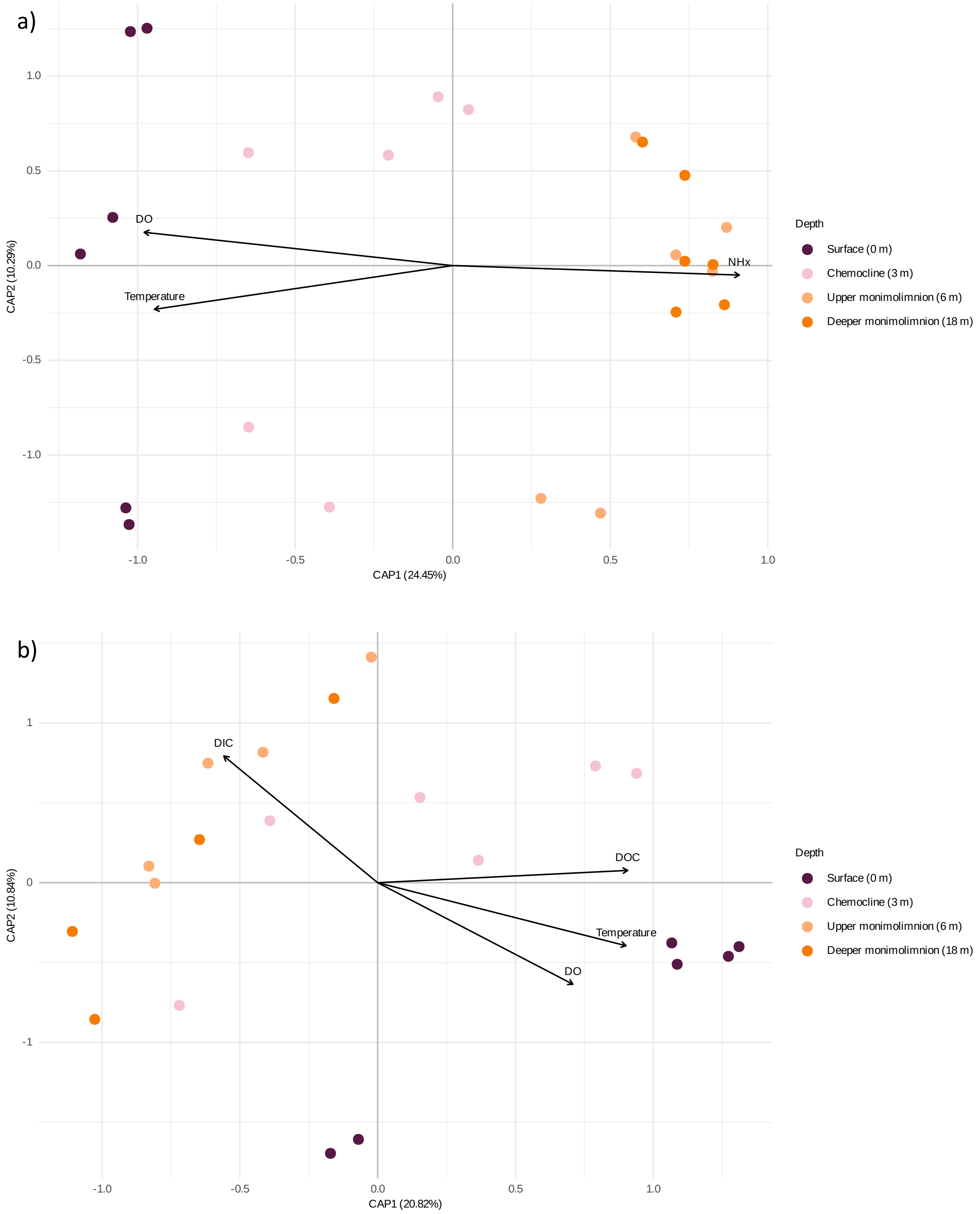

Figure 3. Visualization of the distance-based redundancy analysis (db-RDA) for the bacterial (a) and eukaryotic (b) domains. Arrows represent environmental variables significantly associated with community composition ($p < 0.05$). DIC, dissolved inorganic carbon; DOC, dissolved organic carbon; DO, dissolved oxygen; $NO_3^-$ , nitrates; $NH_x$, ammonia and ammonium.

Variance partitioning quantified the contribution of each significant abiotic factor to microbial community composition (Table S10). In bacterial communities, dissolved oxygen explained 5.7% of the variation, temperature 5.4%, and $NH_x$ 3.5%. In eukaryotic communities, DIC + DOC explained 7.0% of the variation, temperature 4.7%, and dissolved oxygen 0.6%.

The ANOVA by contrast on the Bray-Curtis dissimilarity index performed on bacterial daytime samples revealed that Cu, Fe, and ΣREEs significantly influenced bacterial communities (SI Table S11). The db-RDA output graphs showed that bacterial communities at the surface (0 m) and 3 m were positively associated with higher Cu concentrations, while at 6 and 18 m, bacterial communities were positively related to higher ΣREE and Fe concentrations (SI Figure S6).

### 1.3.5 *Daphnia pulicaria* $\delta^{13}C$ dietary niche along the depth gradient

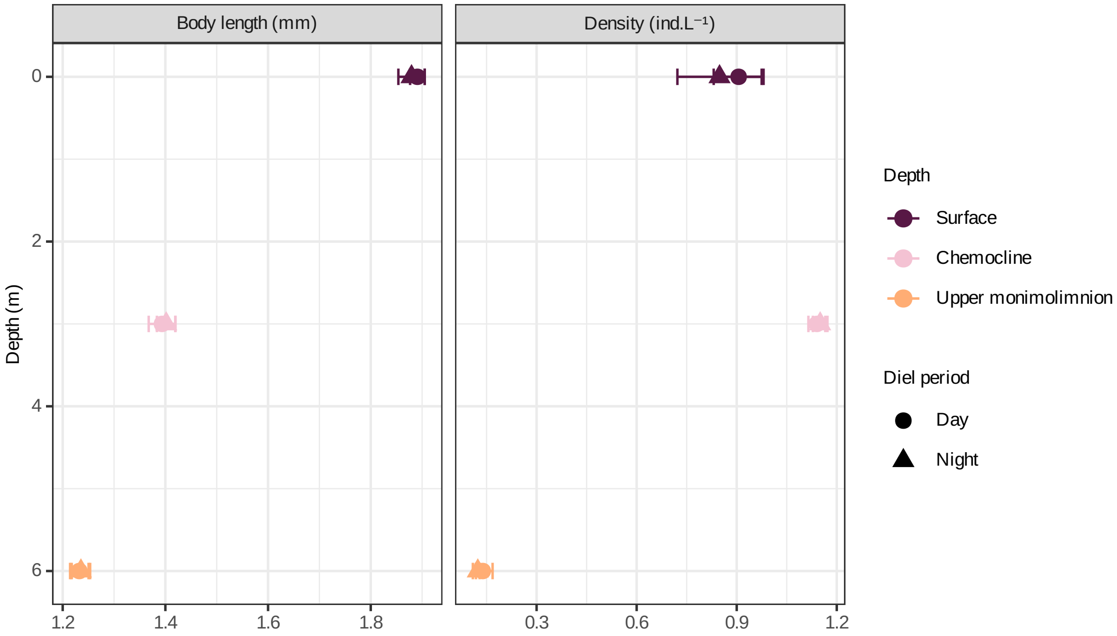

Figure 4. Mean body length and density of *Daphnia pulicaria* across depth and diel periods (day vs night). The errors bars indicate the standard deviation around the mean.

The mean body length of *D. pulicaria* ranged from 1.2 mm to 1.9 mm, while density varied between 0.12 and 1.16 individuals/L (SI Table S12). Larger individuals were observed at the surface compared to deeper depths (3 and 6 m), while the highest *Daphnia* densities were observed at the chemocline (Figure 4), and indeed depth had a significant effect on both body length and density (SI Table S13). However, no significant differences were observed between sampling days or between diel periods at any of the studied depths (SI Table S14).

At the surface, POM $\delta^{13}$C values were between to -36.6 and -33.8‰, while it decreased across depth reaching as low as -51.51‰ at 18 m (Figure 5; SI Figure S7a; SI Table S15). POM $\delta^{13}$C values showed a significant effect of depth (SI Table S16), with significant differences between 0 and 3 m and between 0 and 18 m (SI Table S17). POM $\delta^{15}$N values ranged from -5.0 to 2.6‰ (Figure 5; SI Figure S7b; SI Table S15), and showed significant depth-related variation (SI Table S16), with significant differences observed between 0 and 18 m and between 3 and 18 m (SI Table S17).

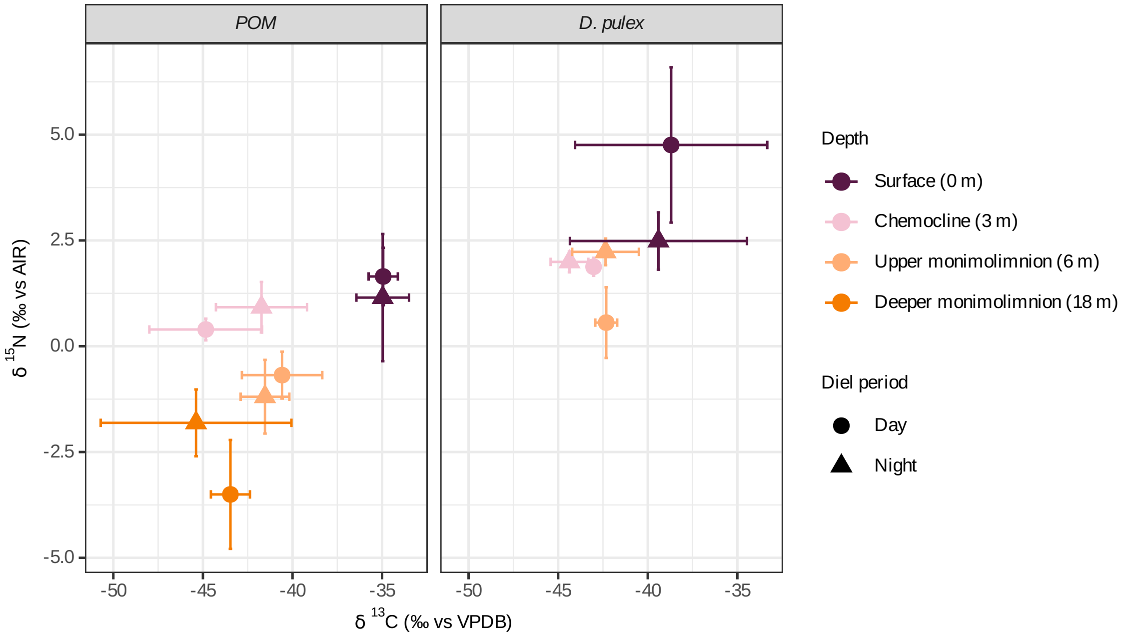

Figure 5. Bi-plot space of $\delta^{13}$C (‰ vs VPDB) and $\delta^{15}$N (‰ vs AIR) in particulate organic matter (POM) and *Daphnia pulicaria* across depth and diel period (day vs night). The error bars indicate the standard deviation of mean.

*D. pulicaria* $\delta^{13}$C values ranged from -45.1 to -34.9‰ (SI Table S15). Although no significant differences in average values were detected among depths (SI Table S16), $\delta^{13}$C values exhibited greater variation at the surface (variance = 17.90) compared to the chemocline (3 m) and upper monimolimnion (6 m) (variance = 1.00 and 1.28, respectively; Figure 5). Across all depths, *D. pulicaria* generally exhibited slightly lower mean $\delta^{13}$ C values compared to POM (SI Figure S7a). For $\delta^{15}$N, Daphnia pulicaria values ranged from 0 to 6.0‰ (Figure 5), and no significant differences were detected among depths (SI Table S16).

No diel differences were observed for $\delta^{13}$C and $\delta^{15}$N values across depths in POM and *D. pulicaria*, with *p*-values greater than 0.05 (SI Table S18). Similarly, no differences were detected between sampling days for both POM (SI Table S19) and *D. pulicaria* (SI Table S20).

In the case of POM, the $\delta^{13}C$ ratios were correlated with $NH_x$ and DIC, with depth, DOC, dissolved oxygen, and $\delta^{15}N$, and with temperature. Meanwhile, $\delta^{15}N$ exhibited strong correlations with depth, temperature, dissolved oxygen, DIC, DOC, and $NH_x$. For *D. pulicaria*, $\delta^{13}C$ showed a significant correlation with temperature and a strong correlation with DOC, while $\delta^{15}N$ was correlated with depth, dissolved oxygen and DIC and *D. pulicaria* body length (SI Figure S8).

## 1.4 Discussion

Our study aimed to explore the basal microbial dietary resource communities and dietary niche of a *Daphnia pulicaria* population in a meromictic mining pit lake. We sought to evaluate how the diversity, community composition, and isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of *D. pulicaria* food resources varied in response to abiotic factors across the depth gradient and over short time periods, both diurnally and throughout the early summer. We examined how the dietary niche of *D. pulicaria* changed across depths in relation to variations in available resources.

Dietary niche variation is commonly evaluated through dietary diversity, which represents the richness and evenness of resources consumed. However, combining this conventional approach with the variance of tissue stable isotope ratios, particularly nitrogen and carbon, provides a more integrated perspective, capturing information on only assimilated prey over time (Bearhop et al., 2004). Using 16S/18S rRNA gene sequencing, we found that while *D. pulicaria*'s food resources alpha-diversity remained relatively stable across depths, depth-related shifts in microbial community composition and $\delta^{13}C$ and $\delta^{15}N$ signatures of POM reflected a transition from photosynthetically derived carbon at the surface to deeper carbon sources likely influenced by the dominance of green sulfur bacteria and methanotrophic activity at the chemocline, and chemosynthetic use of respired $CO_2$. Additionally, *D. pulicaria*'s C-isotopic dietary niche was more variable in the mixolimnion, likely due to higher availability of resources available for foraging. As expected, *D. pulicaria* did not exhibit DVM, likely due to anoxic conditions in the monimolimnion and the absence of fish predation. Instead, individuals remained predominantly in the oxygenated mixolimnion and the chemocline, where food resources were probably more abundant.

Our findings align with previous studies that demonstrated vertical stratification in microbial communities across meromictic systems (Edberg et al., 2012; Oikonomou et al., 2014; Wilk-Woźniak & Żurek, 2006), where shifts in oxygen, light, and chemical composition strongly shape microbial assemblages and basal resource availability. However, our study uniquely integrates both prokaryotic and eukaryotic microbial data with $\delta^{13}C$ and $\delta^{15}N$ isotopic information to assess not only the vertical structuring of basal resources, but also their influence on higher trophic levels. While previous work has often focused on microbial diversity or geochemical patterns independently, our study is, to our knowledge, the first to investigate how depth-related microbial shifts affect the trophic niche and vertical distribution of a primary consumer in a meromictic pit lake. By linking microbial community composition, isotopic signals, and zooplankton ecology over short temporal and vertical scales, we contribute new insight into the functional connections between microbial and zooplanktonic compartments in these chemically and physically stratified environments.

### 1.4.1 Abiotic parameters along the depth gradient in the mining pit lake

The observed abiotic gradients in the Blackburn mine pit lake exhibited typical stratification patterns observed in other meromictic systems, with clear correlations between depth and temperature and dissolved oxygen (Boehrer & Schultze, 2008; Gulati et al., 2017). While previous studies reported an increase in dissolved organic carbon (DOC) with depth in meromictic lakes (Ayala-Muñoz et al., 2022; Čanković et al., 2019), our study revealed a decrease in DOC concentrations with depth, indicating a potential depletion of surface DOC in deeper waters, likely driven by microbial activities (D. Li et al., 2012). In contrast, DIC and $NH_x$ concentrations increased with depth, indicating active microbial remineralization and the dominance of anaerobic metabolic pathways in the monimolimnion. The absence of $NO_2^-$ and the depletion of $NO_3^-$ at greater depths further suggest the reliance on anaerobic respiration processes such as denitrification and sulfate reduction (Castro et al., 1999). Furthermore, the low copper (Cu) concentrations observed in the monimolimnion, compared to the mixolimnion and chemocline, likely reflect the removal of Cu through metal sulfide precipitation (Diez-Ercilla et al., 2014). Iron (Fe) concentrations, however, were lower in the oxic zone and higher in the anoxic zone, as also reported in other meromictic pit lakes (Szarek-Gwiazda & Żurek, 2006). This pattern is consistent with the precipitation of iron oxides under oxic conditions and their subsequent reductive dissolution under anoxia, which releases soluble ferrous iron into the water column. Finally, although previous studies on acidic pit lakes reported only a slight enrichment of REE in surface waters compared to deeper layers (Bozau et al., 2004; Gammons et al., 2003), the Blackburn mine pit lake exhibited a greater accumulation of REE at depth, possibly due to their association with particulate matter and subsequent release under anoxic and reducing conditions.

### 1.4.2 Microbial basal resource communities

At the surface, bacterial communities were dominated by *Proteobacteria*, *Actinobacteriota*, and *Bacteroidota* phyla, which have already been reported in oxic waters of open-pit mining lakes (Edberg et al., 2012). Eukaryotic communities exhibited high temporal variability, but *Chlorophyta* and *Stramenopiles* remained present in the well-lit, oxygenated surface, consistent with previous studies (Oikonomou et al., 2015; Pan et al., 2023). At the chemocline (3 m), bacterial communities continued to be dominated by *Proteobacteria*, *Actinobacteriota*, and *Bacteroidota* phyla, suggesting a gradual transition into the chemocline. Eukaryotic communities at this depth exhibited both diel and seasonal variability, with shifts in community composition potentially influenced by environmental factors such as light availability or nutrient inputs. For instance, *Cryptomonas*, a mixotrophic genus typically found near the oxic–anoxic boundary of the water column (Knapp et al., 2003), was consistently prevalent in this zone but showed fluctuations in relative abundance across day-night cycles and between sampling periods. This genus likely plays an important role in consuming bacterial biomass at the chemocline, where both aerobic and anaerobic processes interact (Barkhatov et al., 2022). In the upper monimolimnion (6m), bacterial communities saw an increase in *Campylobacterota* and *Desulfobacterota* phyla, marking the transition to more reductive conditions. Indeed, the presence of sulfate-reducing bacterial genus, such as *Desulfobulbus*, and *Chlorobium*, a green sulfur bacterium, reflects the shift toward sulfate reduction in this anoxic zone. Additionally, the detection of *Polynucleobacter* in anoxic layers is consistent with its known tolerance to such conditions, as previous studies have reported strains of this genus in the permanently anoxic monimolimnion of temperate meromictic lakes (Hahn et al., 2017; Jezberová et al., 2010). Eukaryotic communities in this layer exhibited an increase in *Cryptomonas*. These algae can migrate into anoxic waters to escape predation by aerobic ciliates (Khromechek et al., 2010) in the mixolimnion, and still consume bacterial biomass below the chemocline. Deeper into the monimolimnion (18 m), bacterial communities were dominated by *Proteobacteria*, *Bacteroidota*, *Campylobacterota*, and *Desulfobacterota*, with the apparition of methanotrophic andmethylotrophic C1-utilizing genera such as *Methylobacter* and *Methylotenera*. This reflects the continued dominance of sulfate-reducing and methane-oxidizing processes in this anoxic environment (Cabello-Yeves et al., 2023; Kallistova et al., 2019; Keshri et al., 2018). Moreover, the presence of *Desulfobulbus*, capable of reducing $NO_3^-$ and $NO_2^-$ to $NH_4^+$ (Dong et al., 2024), and unc. *Arcobacteraceae*, whose family possesses denitrification genes, could explain both depletion of $NO_3^-$, and the observed increase in $NH_x$ concentrations with depth (J. Li et al., 2024). Eukaryotic communities at 18 m were dominated by *Rozellomycota*, the most abundant genus, which has been reported as positively correlated with $NH_4^+$ concentrations in the study by Zhang et al. (2021). Overall, microbial communities across the water column clearly reflected the abiotic gradients, with a transition from oxic to anoxic environments in the deeper waters. This shift was accompanied by a change in microbial metabolism, transitioning from aerobic processes in the surface waters to anaerobic processes such as sulfate reduction and methane cycling in the deeper monimolimnion.

The gradual decline in bacterial Shannon indices across the mixolimnion and chemocline, followed by a resurgence in the deeper monimolimnion, aligns with findings from a previous study on a meromictic pit lake (Andrei et al., 2015), and may reflect the transition from oxygenated surface waters to more specialized anaerobic niches at depth that support diverse metabolic strategies. The only significant difference in bacterial Shannon indices was observed between the mixolimnion and the upper part of the monimolimnion, highlighting a shift in bacterial diversity at the transition from oxygenated to anoxic conditions. However, neither the observed richness and the Pielou's evenness of the bacterial domain nor the alpha diversity indices for the eukaryotic domain exhibited significant variation across depths, which contrasts with studies suggesting that microbial diversity is primarily shaped by depth-related environmental conditions (Čanković et al., 2019).

Despite the lack of a significant effect of depth on most microbial alpha diversity indices, depth (and Cu concentration?) remained a strong structuring factor for community composition. Indeed, PCoA plots revealed a clear separation between microbial communities in the mixolimnion and chemocline, and those in the monimolimnion, for both bacterial and eukaryotic domains. These results align with previous studies of meromictic systems, which reported a complete shift in community structure between the oxic mixolimnion and the anoxic monimolimnion (Andrei et al., 2015; Barberán & Casamayor, 2011; Comeau et al., 2012; Danza et al., 2018; Humayoun et al., 2003; Klepac-Ceraj et al., 2012; Lauro et al., 2011). Moreover, eukaryotic communities in the mixolimnion and deep monimolimnion formed distinct clusters, while communities at 3 m and 6 m were more dispersed. This suggests that the chemocline (3 m) and the transition between oxygenated and anoxic zones in the monimolimnion (6 m) represent intermediate environmental conditions, where microbial communities may experience greater variability in response to fluctuating factors such as oxygen, light availability, and nutrient concentrations.

Although no significant intra-seasonal changes in abiotic parameters were observed during early summer, microbial community composition was influenced by sampling day. This suggests that temporal variability, potentially influenced by unmeasured intra-seasonal factors such as phosphorus inputs from rainfall events, also contributes to shaping microbial assemblages. In contrast, diel period did not significantly influence either community structure, which contradicts the literature, as day-to-night community shifts have been reported for both domains (He et al., 2024; Matyugina et al., 2018), possibly because strong stratification and stable redox conditions in the pit lake reduced the impact of short-term light fluctuations on microbial assemblages. Moreover, the abiotic parameters influencing microbial community composition varied between bacterial and eukaryotic domains. In the mixolimnion and chemocline, bacterial communities were primarily shaped by temperature and DO, while in the monimolimnion, $NH_x$ became the dominant driver. Eukaryotic communities in the upper layers were mainly influenced by temperature, DO, and DOC, whereas deeper eukaryotic communities were more strongly associated with $NH_x$. These patterns suggest that microbial communities structure respond to environmental gradients in a depth-dependent manner, with oxygen and dissolved oxygen availability playing a stronger role in surface layers, while ammonium and ammonia become more relevant in deeper, anoxic conditions. Finally, bacterial communities from the monimolimnion were strongly associated with iron concentrations, which aligns with the presence of *Desulfobacterota*, a phylum known to utilize iron, among other terminal electron acceptors, for respiration or disproportionation processes (Murphy et al., 2021).

### 1.4.3 *Daphnia pulicaria* $\delta^{13}C$ dietary niche along the depth gradient

The isotopic composition of *Daphnia pulicaria* and particulate organic matter (POM) revealed distinct depth-related carbon dynamics in the Blackburn pit lake. Contrary to the literature, which generally reports heavier POM $\delta^{13}C$ values at the surface and a progressive depletion only down to the chemocline (Phillips et al., 2021; Pimenov et al., 2008; Savvichev et al., 2014), our results showed that the decrease in $\delta^{13}C$ continued into the monimolimnion, reaching particularly negative values (as low as –51.5‰), suggesting a transition from photosynthetically derived organic matter at the surface to a strong contribution of microbially derived carbon in these deeper layers. The depleted signal in the monimolimnion may be related to the abundance of the anaerobic photosynthetic bacteria genus *Chlorobium* in this layer, as substantial changes in the carbon isotopic composition of suspended organic matter have been associated with microbial processes carried out by *Chlorobium* spp (Velinsky & Fogel, 1999). This highly negative $\delta^{13}C$ signal in the deeper monimolimnion is likely a result of methanotrophic microbial activity, which preferentially assimilates $^{12}C$, leaving a distinct depletion of $\delta^{13}C$ in the particulate organic matter (Whiticar, 1999), but since archaeal communities were not sequenced, the presence of methanogens cannot be confirmed. The strong correlation between POM $\delta^{13}C$ and dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and oxygen further supports microbial processing as a key driver of carbon fractionation.

*D. pulicaria* $\delta^{13}C$ values were variable in the surface waters, suggesting a diverse range of carbon sources, likely driven by phytoplankton-derived organic matter. In contrast, lower variation at greater depths pointed to a more homogeneous, microbially processed carbon source. Reduced variation of $\delta^{13}C$ signature at depth may indicate a narrowing of *D. pulicaria*'s dietary niche in deeper layers, where limited food sources may restrict dietary flexibility. These results partially support the hypothesis that *D. pulicaria* would exhibit depth-specific $\delta^{13}C$ signatures, with phytoplankton-enriched signals at the surface and microbially derived, $\delta^{13}C$-depleted signals at greater depths. The lack of statistically significant differences between average values for each depth suggests that while carbon source dynamics vary with depth, they may not be distinct enough to produce clear isotopic separation in *D. pulicaria* tissues. However, our findings are based on aggregate isotopic values of POM, and we were unable to distinguish phytoplankton from bacterial sources in the POM using mixing models, which limited the resolution of our trophic interpretation of *D. pulicaria* $\delta^{13}C$ values (Post, 2002). *D. pulicaria* $\delta^{13}C$ values were slightly lower than those of POM, consistent with findings that zooplankton are generally $^{13}C$-depleted relative to smaller planktonic size fractions (Del Giorgio & France, 1996). Additionally, the correlation between *D. pulicaria* $\delta^{13}C$ and temperature suggests that carbon assimilation may be influenced by thermal conditions, likely due to temperature-dependent $CO_2$ solubility, which affects isotope fractionation during phytoplankton uptake (Grey et al., 2001; Rau et al., 1989, 1992).

Nitrogen isotopes revealed depth-related shifts in POM, while *D. pulicaria* $\delta^{15}N$ remained relatively stable across depths. Lower $\delta^{15}N$ values of POM in the monimolimnion were likely due to isotopic fractionation associated with the assimilation of ammonium ($NH_4^+$) into microbial biomass by chemoautotrophic organisms such as sulfide-, methane-, and ammonia-oxidizing microbes (Hadas et al., 2009). *D. pulicaria* consistently exhibited higher $\delta^{15}N$ values than POM, in line with the expected trophic enrichment that occurs between prey and predators (Minagawa & Wada, 1984; Post, 2002; Vander Zanden & Rasmussen, 1999). The strong correlation between $\delta^{15}N$ and *D. pulicaria* body size suggests that larger individuals occupied a slightly higher trophic position. This pattern could reflect differences in local food availability or environmental conditions as temperature or resource quality rather than strict ontogenetic dietary shifts. While surface populations were larger on average, their $\delta^{13}C$ signatures indicated reliance on primary production, making it unlikely that they were consistently feeding on higher trophic levels. An alternative explanation is that larger individuals may have incorporated a greater proportion of microbial eukaryotes or protists, although direct evidence for this in our dataset is lacking.

Unlike in many freshwater systems where *Daphnia* migrate downward during the day to avoid visual predators and return to surface layers at night to feed (Ringelberg, 1991; E. S. Zadereev et al., 2017; Zaret & Suffern, 1976), no significant diel differences in density, body size, or isotopic values were detected across depth layers in our meromictic study lake. Indeed, while Boronat & Miracle (1997), observed that *Daphnia* in a saline meromictic lake aggregated near the surface at night and descended into the mixolimnion during the day in early summer, our results showed maximum densities at the chemocline, minimal presence in the monimolimnion, and no diel variations in densities. Although they also reported a pronounced Daphnia body size gradient with depth, we found the opposite pattern, with larger individuals aggregating near the surface and smaller individuals in higher density at greater depths. The negative correlation between body size and depth suggests that higher food availability in surface waters supports increased birth rates, encouraging reproductive individuals to remain in the mixolimnion (Murtaugh, 1985). Moreover, elevated concentrations of iron and rare earth elements (ΣREE) at greater depths may impair reproduction, further discouraging vertical migration of reproductive individuals (Dave, 1984; Galdiero et al., 2019; Ma et al., 2016). The absence of diel vertical migration (DVM) can be explained by the anoxic conditions of the monimolimnion, restricting habitable oxygenated layers to the mixolimnion (E. S. Zadereev et al., 2017), as well as the lack of fish predation (Beklioglu et al., 2008; Dini & Carpenter, 1992). Our results indicate that *D. pulicaria* have very limited diel vertical migration, and the separation of their $\delta^{13}C$ signatures by depth suggests that individuals remain largely associated with discrete layers of the water column. This restricted overlap with POM $\delta^{13}C$ indicates that surface and deeper individuals may rely on distinct basal resources associated with different depths, which could influence carbon and nitrogen fluxes within the lake. By predominantly residing near the surface, *D. pulicaria* may exert localized grazing pressure on phytoplankton and microbial communities, thereby shaping primary production dynamics.

## 1.5 Conclusion

This study provides novel insights into how environmental depth gradients influence the basal microbial resource communities and the resulting dietary niche of a keystone grazer, *Daphnia pulicaria*, in a meromictic mining lake. Based on 16S/18S rRNA gene sequencing, we observed that the diversity of food resources available to *Daphnia pulicaria* remained relatively across depth. However, variation in microbial community composition and in the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of particulate organic matter (POM) along the water column indicated a shift from surface-derived photosynthetic carbon to deeper carbon sources likely influenced by green sulfur bacteria, methanotrophy at the chemocline, and chemosynthetic pathways fueled by respired $CO_2$. Furthermore, *D. pulicaria* exhibited a more variable carbon isotopic dietary niche in the mixolimnion, likely reflecting higher diversity and availability of food sources in this water stratum. *D. pulicaria* had limited to no diel vertical migration, probably due to the anoxic conditions prevailing in the monimolimnion and the absence of visual predators such as fish. Instead, individuals remained primarily above the monimolimnion, where food was presumably more accessible. Our findings highlight the complex interplay between environmental factors, microbial processes, and zooplankton trophic dynamics in meromictic mining lakes. Future research could explore seasonal and interannual variations in *D. pulicaria* trophic dynamics to assess whether these patterns persist under changing abiotic conditions in meromictic pit lakes influenced by past mining activities. Finally, integrating compound-specific isotope analyses and metagenomic approaches could refine our understanding of microbial contributions to carbon cycling and trophic interactions in meromictic pit lakes. By shedding light on the ecological processes governing these unique ecosystems, our study contributes to a broader understanding of food-web dynamics in abandoned mining lakes and their potential for biogeochemical cycling and ecosystem resilience.

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# CHAPITRE 2

# Discussion générale

Dans cette étude, nous avons examiné la niche trophique d'une population de *Daphnia pulicaria* vivant dans un lac minier submergé de type méromictique situé à Val-des-Monts, au Québec, Canada. L'objectif général était d'évaluer comment les variations des paramètres abiotiques influencent la niche trophique et les dynamiques des sources de carbone de *D. pulicaria* à travers un gradient de profondeur, en tenant compte des variations entre le jour et la nuit et au cours du début de l'été. Les objectifs spécifiques de cette étude étaient d'évaluer comment la diversité, la composition des communautés et les signatures isotopiques ($\delta^{13}C$ et $\delta^{15}N$) des communautés microbiennes servant de ressources alimentaires à *Daphnia pulicaria* variaient en réponse aux facteurs abiotiques le long du gradient de profondeur et sur de courtes périodes de temps, tant diurnes qu'au cours du début de l'été. L'étude visait également à examiner la largeur de la niche trophique de *D. pulicaria* en fonction des différences dans les ressources alimentaires disponibles à travers ce gradient de profondeur, et à étudier les motifs de migration verticale diurne de *D. pulicaria* en relation avec sa niche trophique. En relation avec ces objectifs, nous avons formulé trois hypothèses : que les variations abiotiques le long du gradient de profondeur, entre les périodes jour/nuit et au cours du début de l'été influenceraient la diversité et la composition des communautés microbiennes servant de ressources alimentaires à *D. pulicaria* ; que ces variations influenceraient les signatures isotopiques en $^{13}C$ et en $^{15}N$ de la matière organique particulaire ; et qu'en l'absence de prédateurs visuels et compte tenu des contraintes abiotiques, *D. pulicaria* limiterait ses déplacements verticaux et présenterait des signatures isotopiques $^{13}C$ et en $^{15}N$ dépendantes de la profondeur.

Pour ce faire, nous avons effectué le séquençage amplicon des gènes rRNA 16S et 18S des communautés bactériennes et eucaryotes, respectivement, présentes dans la matière organique particulaire. Nous avons également analysé les rapports isotopiques en $^{13}C$ et $^{15}N$ de la matière organique particulaire ainsi que de *Daphnia pulicaria*. En effet, bien que la largeur de la niche trophique soit généralement évaluée par la diversité alimentaire, qui représente la richesse et l'équité des ressources consommées, combiner cette approche conventionnelle avec la variance des rapports isotopiques des tissus, en particulier du carbone et de l'azote, offre une perspective plus intégrée, capturant des informations uniquement sur les proies assimilées au fil du temps (Bearhop et al., 2004).

Les gradients des paramètres abiotiques observés dans le lac minier Blackburn ont présenté des modèles typiques de stratification observés dans d'autres systèmes méromictiques, avec des corrélations claires entre la profondeur et la température et l'oxygène dissous (Boehrer & Schultze, 2008; Gulati et al., 2017). Tandis que des études précédentes ont signalé une augmentation du carbone organique dissous (COD) avec la profondeur dans les lacs méromictiques (Ayala-Muñoz et al., 2022; Čanković et al., 2019), notre étude a révélé une diminution des concentrations de COD avec la profondeur, indiquant une possible déplétion du COD de surface dans les eaux profondes, probablement causée par les activités microbiennes (D. Li et al., 2012) En revanche, les concentrations de carbone inorganique dissout (CID) et de $NH_x$ ont augmenté avec la profondeur, suggérent une reminéralisation microbienne active et la dominance de voies métaboliques anaérobies dans le monimolimnion. L'absence de $NO_2^-$ et la déplétion de $NO_3^-$ dans les couches plus profondes suggèrent en outre la dépendance aux processus respiratoires anaérobies tels que la dénitrification et la réduction du soufre dans le monimolimnion (Castro et al., 1999). De plus, les faibles concentrations de cuivre observées dans le monimolimnion, par rapport au mixolimnion et à la chimiocline, reflètent l'élimination du Cu par précipitation de sulfures métalliques (Diez-Ercilla et al., 2014). Les concentrations de fer étaient plus faibles dans la zone oxique et plus élevées dans la zone anoxique, comme cela a été rapporté dans d'autres lacs minier méromictiques par Szarek-Gwiazda & Żurek (2006). Enfin, bien que des études précédentes sur les lacs miniers acides aient rapporté seulement une légère enrichissement en éléments terres rares dans les eaux de surface par rapport aux couches profondes (Bozau et al., 2004; Gammons et al., 2003), le lac minier Blackburn a montré une grande accumulation de REE en profondeur.

À la surface, les communautés bactériennes étaient dominées par les phylums *Proteobacteria*, *Actinobacteriota* et *Bacteroidota*, déjà reportées dans les eaux oxiques de lacs miniers (Edberg et al., 2012). Les communautés eucaryotes ont montré une grande variabilité temporelle, bien que les phylums *Chlorophyta* et *Stramenopiles* aient persisté dans la zone de surface éclairée et oxygénée à travers le début de l'été, en accord avec d'autres études (Oikonomou et al., 2015; Pan et al., 2023). Au niveau de la chimiocline, les communautés bactériennes ont continué à être dominées par les phylums *Proteobacteria*, *Actinobacteriota* et *Bacteroidota*, suggérant une transition progressive de celles-ci. Les communautés eucaryotes à cette profondeur ont montré une forte variabilité diurne et saisonnière, avec *Cryptomonas*, un genre mixotrophe typiquement trouvé aux abords de la frontière oxique-anoxique de la colonne d'eau, prédominant dans cette zone (Knapp et al., 2003). Ce genre joue probablement un rôle important dans la consommation de biomasse bactérienne au niveau de la chimiocline, où les processus aérobiques et anaérobies interagissent (Barkhatov et al., 2022). Dans la partie supérieure du monimolimnion, les communautés bactériennes ont montré une augmentation des phylums *Campylobacterota* et *Desulfobacterota*, marquant la transition vers des conditions plus réductrices. En effet, la présence de genres bactériens réducteurs de sulfate, tels que *Desulfobulbus*, et de bactéries sulfurées vertes, comme *Chlorobium*, reflète le passage à la réduction du soufre dans cette zone anoxique. De plus, la détection de *Polynucleobacter* dans les couches anoxiques est cohérente avec sa tolérance connue à de telles conditions, comme l'ont rapporté des études précédentes sur des souches de ce genre dans le monimolimnion anoxique de lacs meromictiques tempérés (Hahn et al., 2017; Jezberová et al., 2010). Les communautés eucaryotes du monimolimnion ont montré une augmentation de *Cryptomonas*, capable de migrer dans les eaux anoxiques afin de réduire la prédation (Khromechek et al., 2010), suggérant une consommation continue de biomasse bactérienne sous le chimiocline. Plus profondément dans le monimolimnion, à 18 m, les communautés bactériennes étaient dominées par les phylums *Proteobacteria*, *Bacteroidota*, *Campylobacterota* et *Desulfobacterota*, avec l'apparition de genres méthanotrophes comme *Methylobacter* et *Methylotenera*. Cela reflète la dominance continue des processus réducteurs de sulfate et de méthanotrophie dans cet environnement anoxique (Cabello-Yeves et al., 2023; Kallistova et al., 2019; Keshri et al., 2018). De plus, la présence de *Desulfobulbus*, capable de réduire $NO_3^-$ et $NO_2^-$ en $NH_4^+$ (Dong et al., 2024), et de Unclassified *Arcobacteraceae*, dont la famille possède des gènes de dénitrification, pourrait expliquer à la fois la déplétion de $NO_3^-$ et l'augmentation des concentrations de $NH_x$ avec la profondeur (J. Li et al., 2024). Les communautés eucaryotes à 18 m étaient dominées par *Rozellomycota*, le genre le plus abondant, qui a été rapporté comme étant positivement corrélé avec les concentrations de $NH_4^+$ dans l'étude de Zhang et al. (2021). Dans l'ensemble, les communautés microbiennes à travers la colonne d'eau ont clairement reflété les gradients abiotiques, avec une transition entre les environnements oxique et anoxique. Cependant, il est possible que certains taxons bactériens et eucaryotes aient échappé à la détection, car la filtration a été effectuée sur des filtres de 0.7 µm, une porosité plus élevée que celle de 0.25 µm couramment utilisée. Cette limitation méthodologique importante pourrait avoir biaisé partiellement la représentation des communautés microbiennes observées. De la même façon, le domaine des archées n'a pas pu être séquencé en raison d'une quantité d'ADN insuffisante, probablement liée à cette porosité plus élevée, qui aurait empêché la rétention efficace de ces microorganismes souvent de très petite taille.

Ces structures microbiennes profondément influencées par les gradients des paramètres abiotiques liés à la profondeur ont également influencé les signatures isotopiques de la MOP, reflet indirect de l'origine du carbone assimilé par *D. pulicaria*. En effet, la composition isotopique de *Daphnia pulicaria* et de la matière organique particulaire (MOP) a révélé des dynamiques du carbone liées à la profondeur dans le lac minier Blackburn. Cependant, contrairement à la littérature, qui rapporte généralement des valeurs plus lourdes de $\delta^{13}C$ de la MOP en surface et un appauvrissement progressif jusqu'à la chimiocline (Phillips et al., 2021; Pimenov et al., 2008; Savvichev et al., 2014), nos résultats indiquent que la diminution du $\delta^{13}C$ se poursuit dans le monimolimnion, atteignant des valeurs particulièrement négatives au de celui-ci (jusqu'à –51,5 ‰). Cela suggère une transition entre une matière organique d'origine photosynthétique en surface à une contribution microbienne marquée dans les couches plus profondes. Ce signal fortement appauvri en $\delta^{13}C$ dans le monimolimnion pourrait être associé à l'abondance du genre bactérien photosynthétique anaérobie *Chlorobium* dans cette couche, car des changements significatifs de la composition isotopique du carbone de la matière organique en suspension ont été liés à des processus microbiens assurés par les espèces de *Chlorobium* (Velinsky & Fogel, 1999). Le signal $\delta^{13}C$ extrêmement négatif au fond du monimolimnion est quant à lui probablement lié à l'activité de bactéries méthanotrophes, qui assimilent préférentiellement le $^{12}C$, entraînant un appauvrissement marqué en $\delta^{13}C$ dans la MOP (Whiticar, 1999). La forte corrélation entre le $\delta^{13}C$ de la MOP et le carbone inorganique dissous, le carbone organique dissous et l'oxygène appuie l'hypothèse d'un traitement microbien du carbone comme moteur principal du fractionnement isotopique. Cependant, Il est important de souligner que la matière organique particulaire ne correspond pas directement aux communautés microbiennes vivantes : elle inclut également une fraction détritique importante provenant de matériel organique mort ou en décomposition, qui n'est pas nécessairement représentative des ressources réellement consommées par *Daphnia pulicaria*. Toutefois, dans le contexte particulier des lacs miniers méromictiques, dont les échanges avec les systèmes environnants sont très limités, la MOP peut être considérée comme un indicateur global des communautés microbiennes locales - vivantes ou mortes - et des processus biogéochimiques associés.

La forte variance des valeurs de $\delta^{13}C$ de *D. pulicaria* en surface suggère une diversité des sources de carbone assimilées, probablement dominées par une matière organique d'origine phytoplanctonique. En revanche, la plus faible variation observée en profondeur indique une source de carbone plus homogène et probablement traitée par des microorganismes bactériens. Cette réduction de la variabilité du $\delta^{13}C$ en profondeur pourrait refléter un rétrécissement de la niche trophique de *D. pulicaria* dans les couches profondes, où l'abondance des ressources alimentaires est probablement plus limitée. Ces résultats confirment partiellement notre hypothèse selon laquelle *D. pulicaria* présenterait des signatures isotopiques en $\delta^{13}C$ spécifiques à la profondeur, avec des valeurs enrichies en surface liées au phytoplancton, et des valeurs appauvries plus profondément, reflétant une origine microbienne du carbone. Toutefois, l'absence de différences significatives entre les moyennes de $\delta^{13}C$ selon la profondeur indique que, bien que les sources de carbone et la signature isotopique de la MOP varient avec la profondeur, ces variations ne sont pas assez prononcées pour entraîner une différenciation isotopique nette dans les tissus de *D. pulicaria*. De plus, notre interprétation trophique du $\delta^{13}C$ repose sur des valeurs agrégées de la MOP, sans pouvoir distinguer la contribution respective du phytoplancton et des bactéries à l'aide de mixing models (Post, 2002). Toutefois, les valeurs de $\delta^{13}C$ de *D. pulicaria* étaient légèrement inférieures à celles de la MOP, ce qui est cohérent avec les observations selon lesquelles le zooplancton est généralement appauvri en $^{13}C$ par rapport aux fractions planctoniques plus petites (Del Giorgio & France, 1996). Par ailleurs, la corrélation entre le valeurs de $\delta^{13}C$ de *D. pulicaria* et la température suggère une influence des conditions thermiques sur l'assimilation du carbone, probablement en lien avec la solubilité du $CO_2$, qui module le fractionnement isotopique lors de son assimilation par le phytoplancton (Grey et al., 2001; Rau et al., 1989, 1992).

Les isotopes de l'azote ont révélé des variations liées à la profondeur pour la MOP, tandis que le $\delta^{15}N$ de *D. pulicaria* est demeuré relativement stable. Les faibles valeurs $\delta^{15}N$ de la MOP dans le monimolimnion pourraient être attribuées au fractionnement isotopique associé à l'assimilation de l'ammonium ($NH_4^+$) par la biomasse microbienne, notamment chez les microorganismes chimiotrophes comme ceux oxydant le sulfure, le méthane ou l'ammoniac (Hadas et al., 2009). Toutefois, les valeurs de $\delta^{15}N$ de *D. pulicaria* étaient systématiquement plus élevées que celles de la MOP, conformément à l'enrichissement trophique attendu entre proies et prédateurs (Minagawa & Wada, 1984; Post, 2002; Vander Zanden & Rasmussen, 1999). La forte corrélation entre le $\delta^{15}N$ et la taille corporelle de *D. pulicaria* suggère des changements ontogénétiques dans le régime alimentaire, les individus plus grands occupant un niveau trophique légèrement supérieur. En outre, la corrélation du $\delta^{13}C$ et du $\delta^{15}N$ de la MOP avec la profondeur et le COD renforce l'idée que les transformations microbiennes façonnent les réservoirs de carbone et d'azote disponibles pour les consommateurs primaires tels que *Daphnia pulicaria*.

Contrairement à de nombreux écosystèmes d'eau douce où *Daphnia* migre vers le bas pendant le jour pour éviter les prédateurs visuels, puis remonte à la surface la nuit pour se nourrir (Ringelberg, 1991; E. S. Zadereev et al., 2017; Zaret & Suffern, 1976), aucune différence significative entre le jour et la nuit n'a été détectée dans les densités, les tailles corporelles ou les valeurs isotopiques selon la profondeur. Alors que Boronat & Miracle (1997) ont observé que les daphnies d'un lac salin méromictique s'agrégeaient près de la surface la nuit et descendaient dans le mixolimnion le jour en début d'été, nos résultats montrent une densité maximale au niveau de la chimiocline, une présence minimale dans le monimolimnion, et aucune variation nycthémérale de la densité. De plus, contrairement à leur observation d'un gradient de taille positif avec la profondeur, nous avons observé le schéma inverse, avec des individus plus grands en surface et plus petits en profondeur. La corrélation négative entre la taille corporelle et la profondeur pourrait s'expliquer par une plus grande disponibilité de nourriture en surface, favorisant une forte natalité et incitant les individus reproducteurs à demeurer dans le mixolimnion (Murtaugh, 1985). Par ailleurs, les concentrations élevées de fer et d'éléments terres rares (ΣREE) en profondeur pourraient nuire à la reproduction, décourageant ainsi la migration verticale des individus reproducteurs (Dave, 1984; Galdiero et al., 2019; Ma et al., 2016). L'absence de migration verticale nycthémérale peut s'expliquer par les conditions anoxiques du monimolimnion, qui limitent les couches habitables à la zone oxygénée du mixolimnion (E. S. Zadereev et al., 2017), ainsi que par l'absence de prédation par les poissons (Beklioglu et al., 2008; Dini & Carpenter, 1992). Le déplacement vertical restreint de *D. pulicaria* pourrait avoir des répercussions sur les flux de carbone et d'azote, influençant l'activité microbienne et la décomposition de la matière organique dans le lac. En résidant principalement près de la surface, *D. pulicaria* pourrait exercer une pression de broutage localisée sur les communautés phytoplanctoniques et microbiennes, contribuant ainsi à structurer les dynamiques de production primaire.

# CONCLUSION

Cette étude apporte un nouvel éclairage sur la niche trophique de *Daphnia pulicaria* dans un lac minier méromictique, en mettant en évidence l'influence des gradients environnementaux sur sa position trophique et ses sources primaires de carbone.

À partir du séquençage des gènes 16S/18S rRNA, nous avons observé que, bien que la diversité des ressources alimentaires disponibles pour *D. pulicaria* soit restée relativement constante avec la profondeur, les variations de la composition des communautés microbiennes le long de la colonne d'eau ont révélé une forte influence des gradients abiotiques, notamment de l'oxygène et de la température à la surface, ainsi que de $NH_x$ en profondeur. Grâce aux analyses isotopiques ($\delta^{13}C$ et $\delta^{15}N$), nous avons observé que l'étendue de la niche trophique de *D. pulicaria* ne variait pas significativement selon la profondeur, la période nycthémérale ou les changements intra-saisonniers, suggérant une stratégie alimentaire relativement stable. Les valeurs de $\delta^{13}C$ de *D. pulicaria* étaient systématiquement plus faibles que celles de la matière organique particulaire, tandis que le $\delta^{13}C$ du MOP montrait un appauvrissement marqué en profondeur. Cette signature isotopique témoigne de l'importance du recyclage microbien du carbone et de l'influence des bactéries méthanotrophes dans le monimolimnion. Contrairement aux migrations verticales journalières observées dans de nombreux écosystèmes d'eau douce, *D. pulicaria*, dans ce lac méromictique, est restée confinée aux couches oxiques du mixolimnion, sans différences significatives entre le jour et la nuit en termes de valeurs isotopiques, de taille corporelle ou de densité. Cette absence de migration verticale peut être expliquée par l'anoxie permanente du monimolimnion, ainsi que par l'absence de prédateurs visuels, réduisant ainsi le besoin d'évitement.

Ces résultats contribuent à une meilleure compréhension des dynamiques trophiques dans les lacs miniers méromictiques, où les conditions hostiles influencent les interactions trophiques du zooplancton. Des recherches futures pourraient examiner les variations saisonnières des compositions isotopiques et des communautés microbiennes pour déterminer si ces tendances persistent sur des échelles temporelles plus longues. Une prochaine étude intégrant *Chaoborus*, le niveau trophique supérieur dans ce système exercant une pression de prédation sur *Daphnia pulicaria* plus tard dans la saison, permettrait d'éclairer davantage la dynamique alimentaire, en examinant l'interaction entre les prédateurs et les consommateurs primaires dans ces écosystèmes. En élucidant le rôle écologique des consommateurs primaires dans ces lacs abandonnés, cette étude améliore notre compréhension du cyclage biogéochimique et de la stabilité des écosystèmes des lacs miniers méromictiques.

# ANNEXE A

## Figures supplémentaires

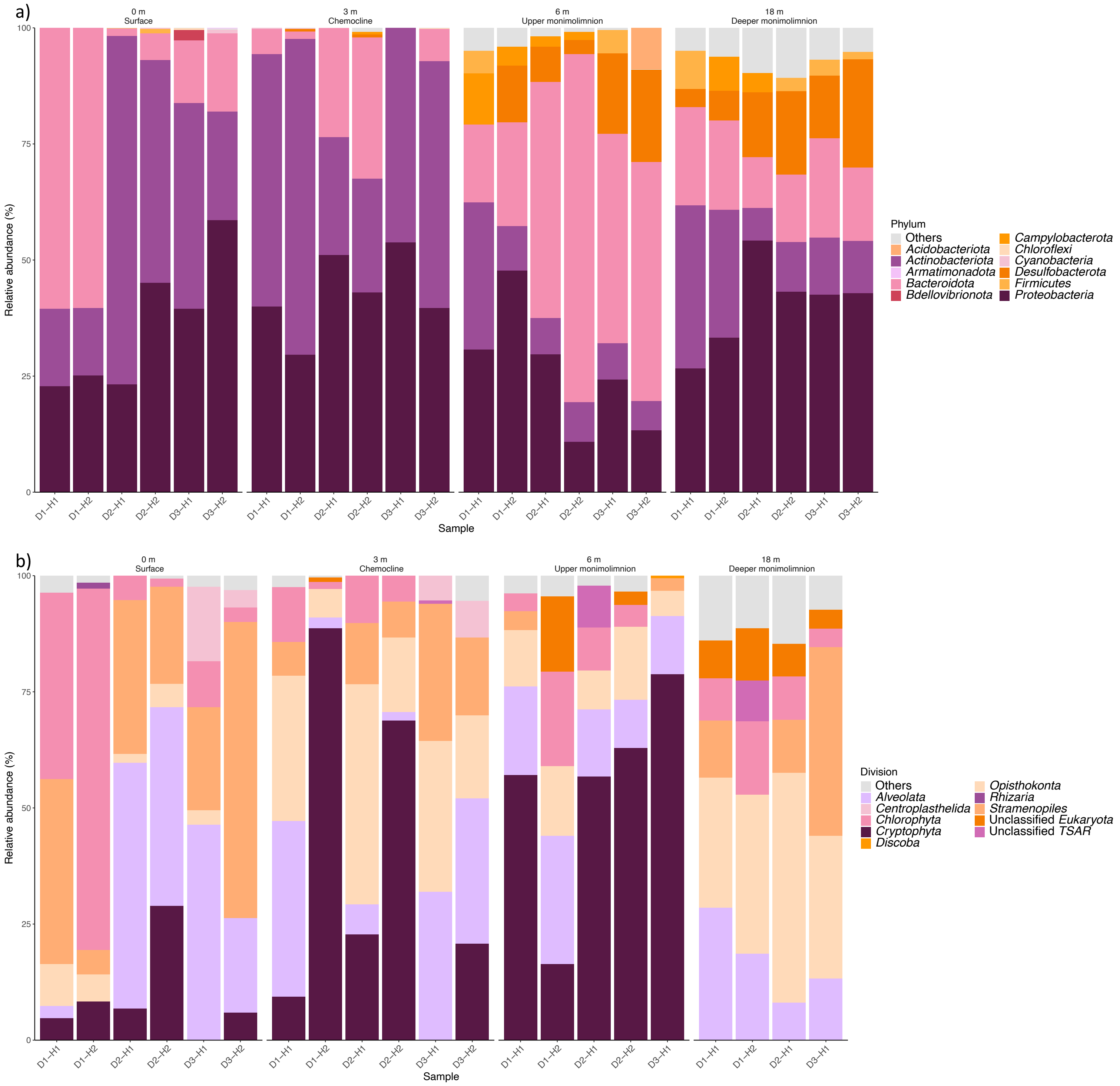

Figure S1. Relative abundance (% of total reads) of bacterial taxa at the phylum level (a) and of eukaryote taxa at the division level (b) across depths, based on 16S and 18S rRNA gene sequencing. D, day; H, hour (H1: Night, H2: Day).

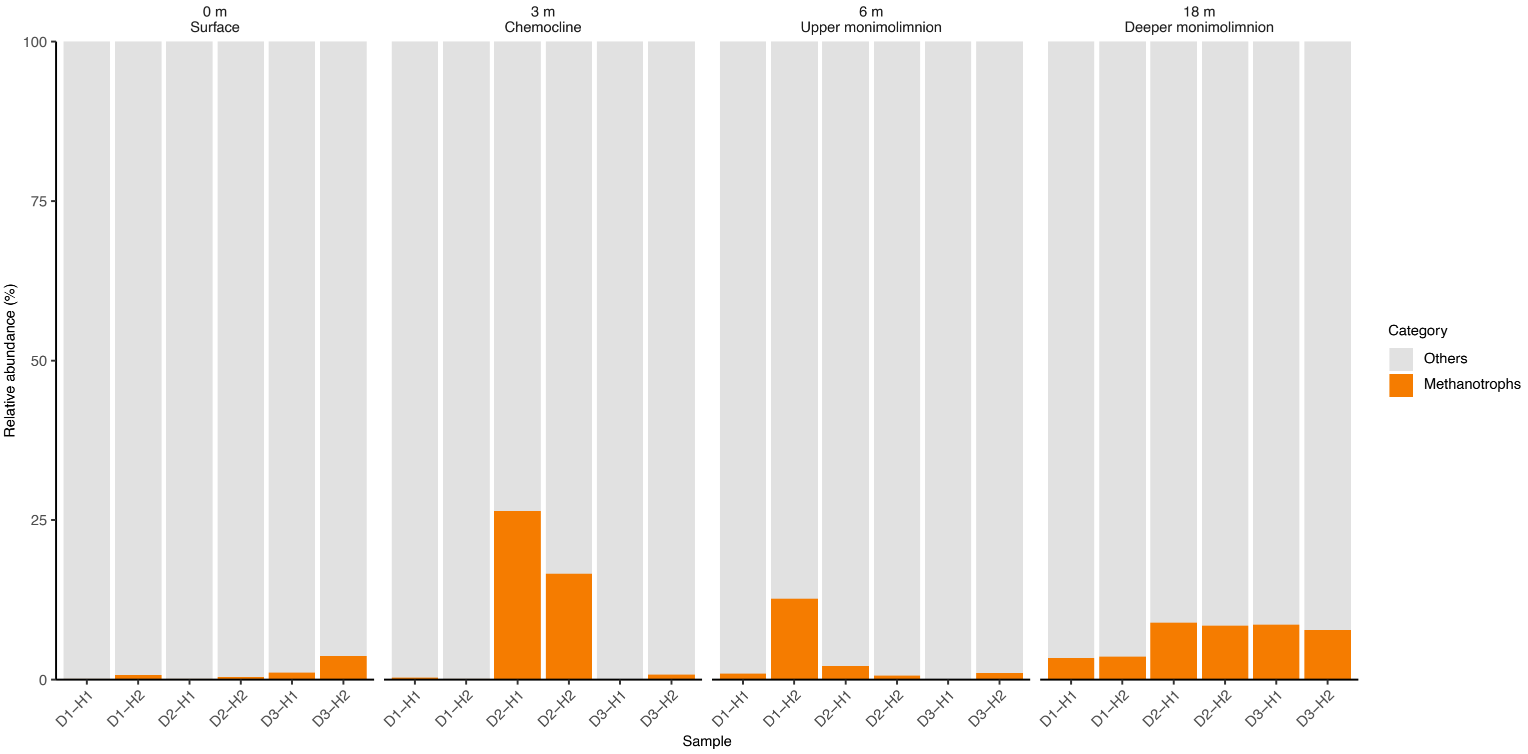

Figure S2. Relative abundance (% of total reads) of methanotrophs across depths, based on 16S and 18S rRNA gene sequencing. D, day; H, hour (H1: Night, H2: Day).

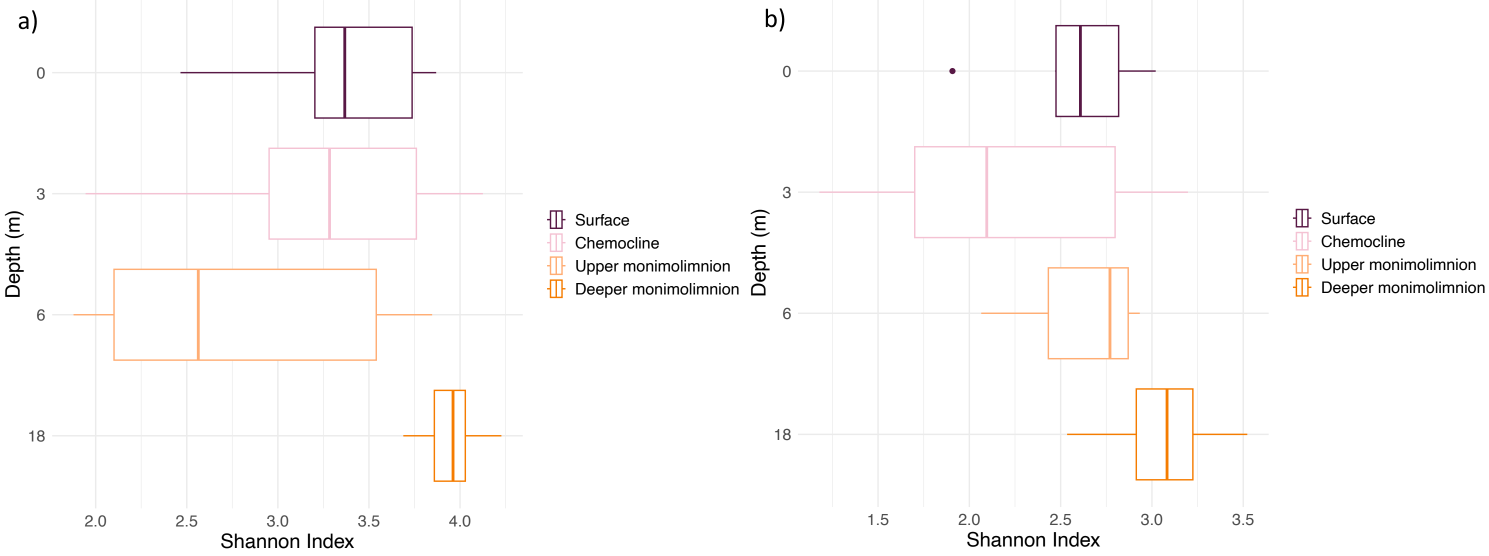

Figure S3. Boxplots showing Shannon Index across depths for bacteria (a) and eukaryote (b) communities.

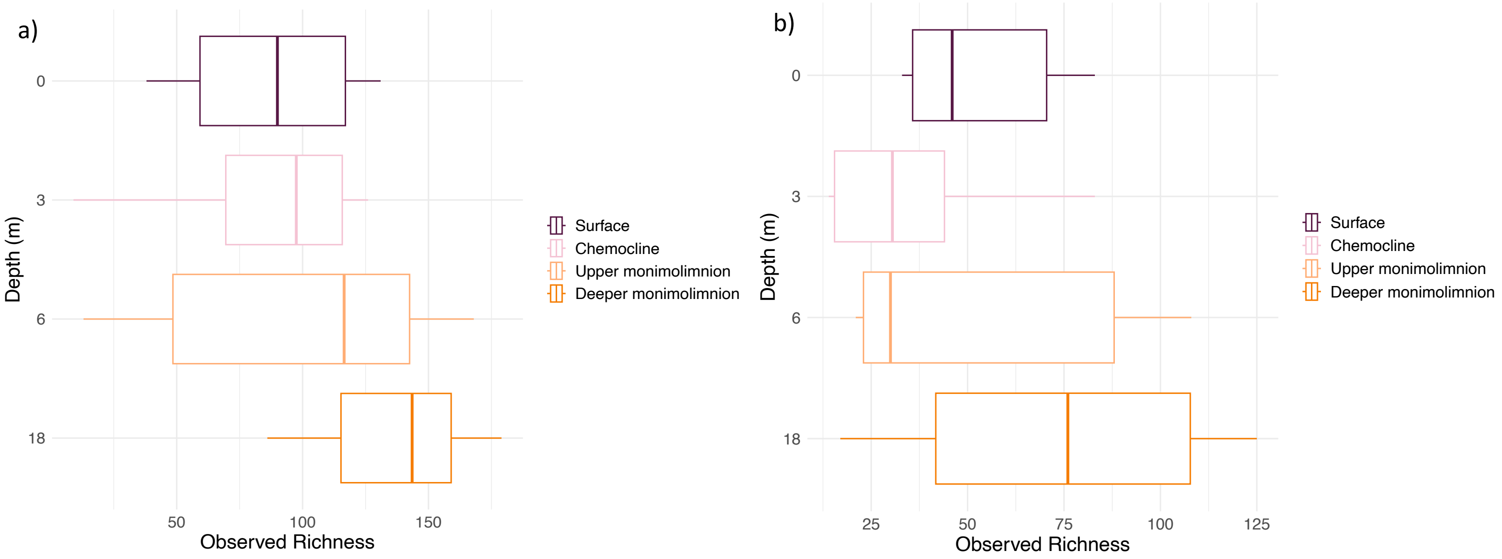

Figure S4. Boxplots showing observed richness across depths for bacteria (a) and eukaryote (b) communities.

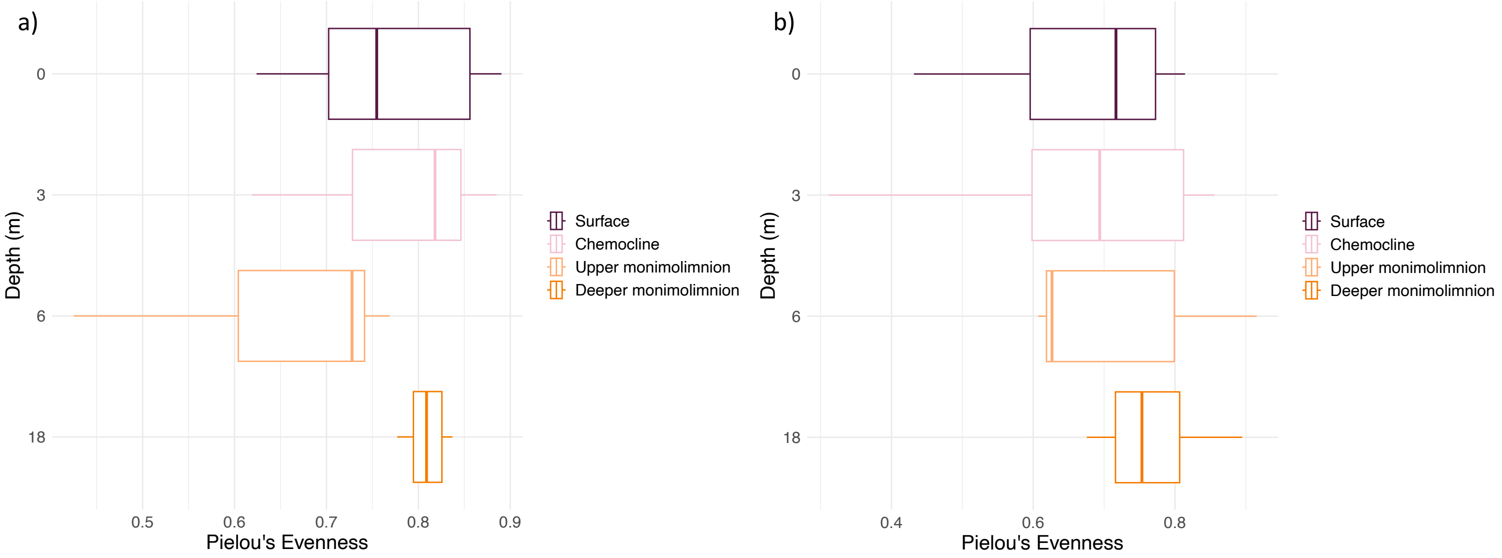

Figure S5. Boxplots showing Pielou’s evenness across depths for bacteria (a) and eukaryote (b) communities..

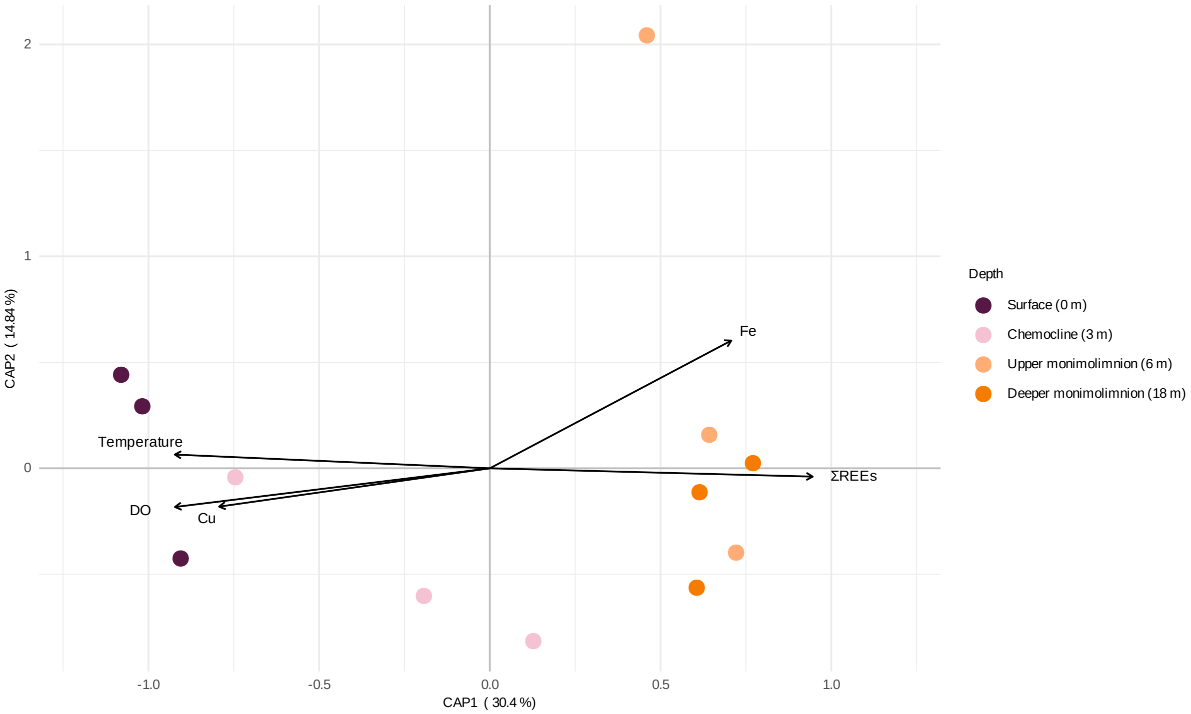

Figure S6. Visualization of the distance-based redundancy analysis (db-RDA) for the bacterial domain, based on samples collected during the day. Arrows represent environmental variables significantly associated with community composition ($p < 0.05$). Cu, copper; DOC, dissolved organic carbon; DO, dissolved oxygen; Fe, Iron; ΣREEs, sum of Rare Earth Elements.

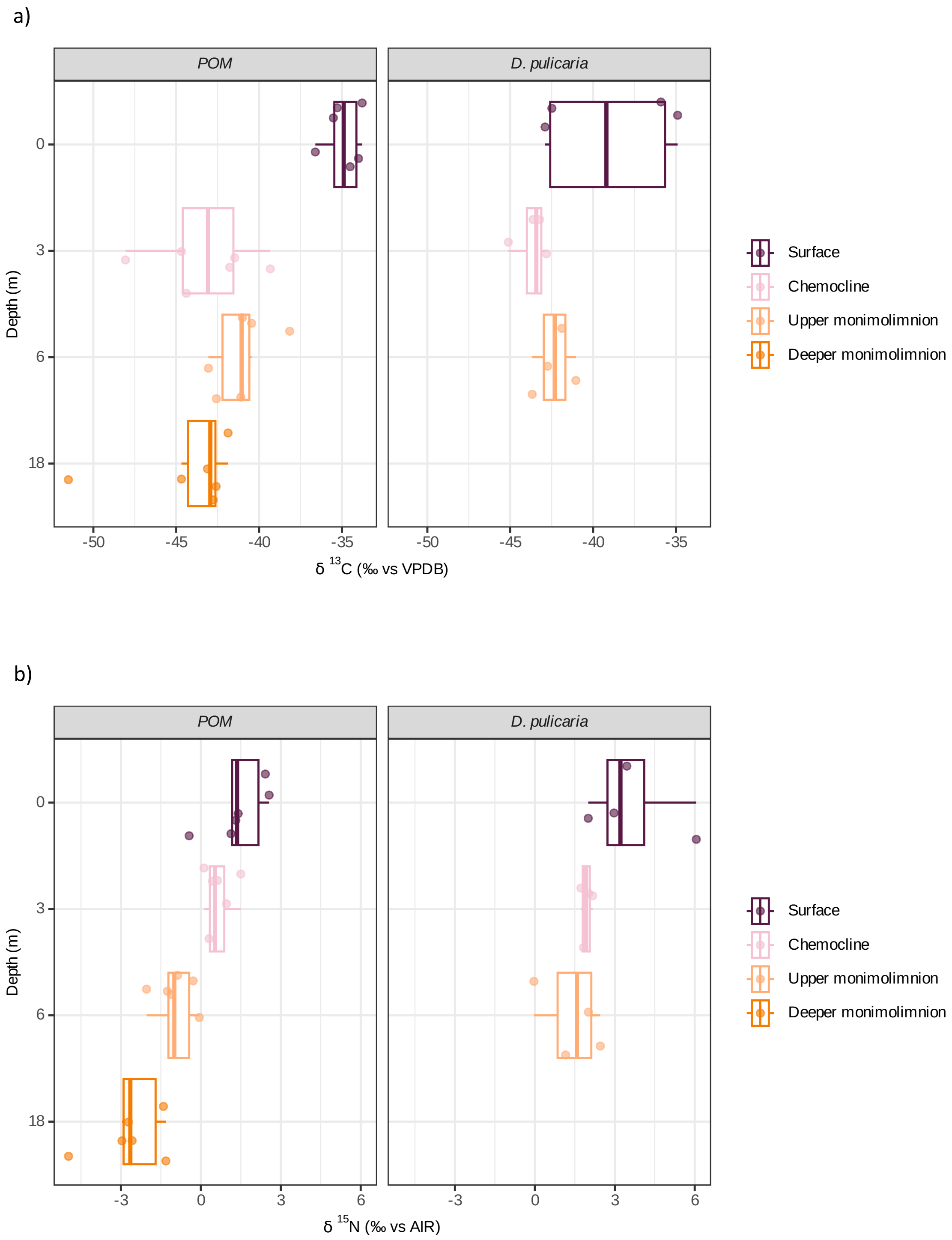

Figure S7. Box plots of δ$^{13}$C (‰ vs VPDB) (a) and δ$^{15}$N (‰ vs AIR) (b) in particulate organic matter (POM) abd Daphnia pulicaria across depth.

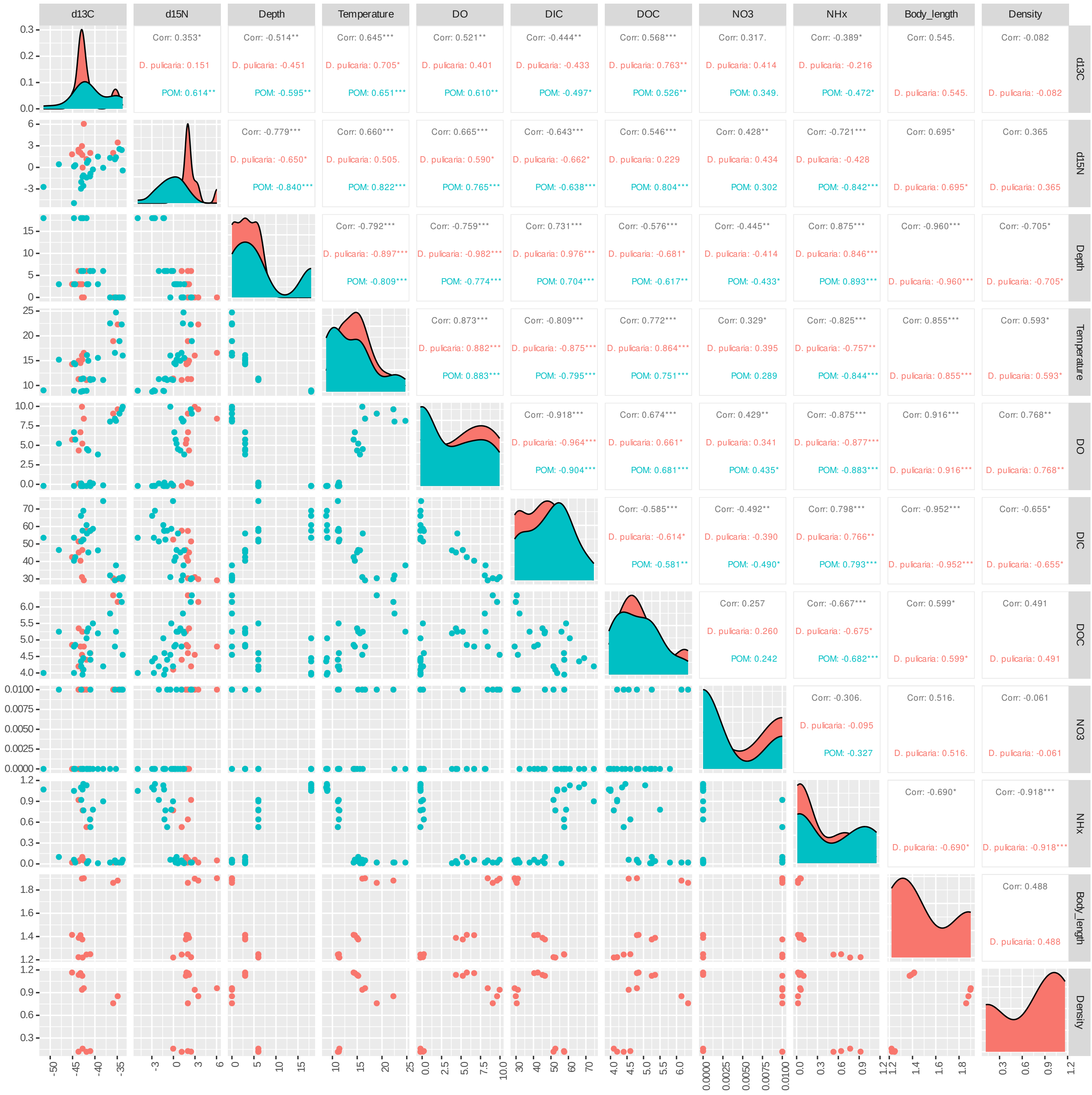

Figure S8. Pair plot displaying Pearson correlations between $\delta^{13}$Cand $\delta^{15}$N and depth or all abiotic parameters, mean body length, and density. ***, p < 0.001; **, p < 0.01; *, p < 0.05; . , p < 0.1. Corr, Pearson correlation coefficients; DO, dissolved oxygen; DIC, dissolved inorganic carbon; DOC, dissolved organic carbon; NO3, nitrates; NHx, ammonia and ammonium.

# ANNEXE B

## Tableaux supplémentaires

Table S1. PCR conditions for the amplification of the 16S/18S rRNA genes.

| Domain | Primers | | Denaturation | Annealing | Final extension |
|---|---|---|---|---|---|
| Bacteria | B341F and B785R | Nb of cycles | 35 | | 1 |
| | | Time (s) | 30 | 60 | 30 |
| | | Temperature (°C) | 98 | 57 | 98 |
| Eukaryote | E960F and NSR1438R | Nb of cycles | 35 | | 1 |
| | | Time (s) | 30 | 30 | 1 |
| | | Temperature (°C) | 98 | 61 | 98 |

Table S2. Abiotic parameters across sampling days, diel periods and depths. DO, dissolved oxygen; DOC, dissolved organic carbon; DIC, dissolved inorganix carbon; $NO_2^-$, nitrites; $NO_3^-$, nitrates; $NH_x$, ammonium and ammonia; Cu, copper; Fe, iron; ΣREEs, sum of rare earth elements.

| Sampling day | Diel period | Depth (m) | Temperature (°C) | DO (mg/L) | DOC (mg/L) | DIC (mg/L) | $NO_2^-$ (mg/L) | $NO_3^-$ (mg/L) | $NH_x$ (mg/L) | Cu (nmol/L) | Fe (nmol/L) | ΣREEs (nmol/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Night | 0 | 16.03 | 9.95 | 4.55 | 31.05 | 0 | 0.01 | 0.06 | | | |
| 1 | Day | 0 | 16.58 | 8.42 | 4.80 | 29.20 | 0 | 0.01 | 0.05 | 16.94 | 193.42 | 1.01 |
| 1 | Night | 3 | 14.08 | 5.73 | 4.85 | 42.45 | 0 | 0 | 0.02 | | | |
| 1 | Day | 3 | 14.52 | 6.68 | 4.80 | 40.40 | 0 | 0 | 0.06 | 6.39 | 592.23 | 2.00 |
| 1 | Night | 6 | 11.28 | 0.11 | 4.20 | 51.45 | 0 | 0.01 | 0.92 | | | |
| 1 | Day | 6 | 11.40 | -0.21 | 4.1 | 52.50 | 0 | 0 | 0.77 | 1.10 | 1629.13 | 4.07 |
| 1 | Night | 18 | 9.02 | -0.20 | 4.00 | 53.55 | 0 | 0 | 1.07 | | | |
| 1 | Day | 18 | 8.95 | -0.20 | 4.00 | 53.55 | 0 | 0 | 1.05 | 0.86 | 1319.69 | 4.10 |
| 2 | Night | 0 | 18.95 | 9.07 | 6.35 | 30.40 | 0 | 0.01 | 0.02 | | | |
| 2 | Day | 0 | 22.30 | 9.63 | 6.15 | 29.85 | 0 | 0.01 | 0.02 | 16.50 | 299.53 | 0.92 |
| 2 | Night | 3 | 14.98 | 4.34 | 5.35 | 45.15 | 0 | 0 | 0.04 | | | |
| 2 | Day | 3 | 15.20 | 5.22 | 5.25 | 46.55 | 0 | 0.01 | 0.10 | 26.31 | 397.11 | 1.38 |
| 2 | Night | 6 | 11.30 | 0.22 | 4.60 | 57.45 | 0 | 0 | 0.64 | | | |
| 2 | Day | 6 | 11.10 | -0.22 | 4.40 | 57.60 | 0.01 | 0.01 | 0.53 | 0.85 | 1802.92 | 3.70 |
| 2 | Night | 18 | 8.86 | -0.21 | 3.95 | 57.6 | 0 | 0 | 1.07 | | | |
| 2 | Day | 18 | 8.74 | -0.22 | 4.35 | 66.10 | 0 | 0 | 1.1 | 0.76 | 1237.81 | 4.19 |
| 3 | Night | 0 | 22.52 | 8.05 | 5.80 | 32.00 | 0 | 0 | 0.02 | | | |
| 3 | Day | 0 | 24.72 | 8.14 | 5.25 | 37.75 | 0 | 0 | 0.02 | 17.92 | 1107.90 | 0.76 |
| 3 | Night | 3 | 15.56 | 3.83 | 5.20 | 46.35 | 0 | 0 | 0.01 | | | |
| 3 | Day | 3 | 16.12 | 4.52 | 5.25 | 56.00 | 0 | 0 | 0.01 | 8.15 | 320.84 | 1.37 |
| 3 | Night | 6 | 11.24 | 0.10 | 5.50 | 58.65 | 0 | 0 | 0.78 | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | Day | 6 | 11.10 | -0.13 | 4.20 | 74.55 | 0 | 0 | 0.90 | 1.64 | 2392.80 | 2.75 |
| 3 | Night | 18 | 8.93 | -0.21 | 5.05 | 60.80 | 0 | 0 | 1.13 | | | |
| 3 | Day | 18 | 8.88 | -0.21 | 4.45 | 69.00 | 0 | 0 | 1.15 | 0.34 | 1230.60 | 4.29 |

Table S3. Spearman correlation between abiotic parameters and depth. For temperature, dissolved oxygen, DOC, DIC, $NO_3^-$, and $NH_x$, n = 6; for Cu, Fe, and ΣREEs, n = 3. Significant values are in bold. DO, dissolved oxygen; DOC, dissolved organic carbon; DIC, dissolved inorganix carbon; $NO_3^-$, nitrates; NHx, ammonium and ammonia; Cu, copper; Fe, iron; ΣREEs, sum of rare earth elements.

| **Abiotic parameter** | **rho (ρ)** | ***p*** |
|---|---|---|
| Temperature | - 0.9614497 | $\mathbf{1.12 \times 10^{-12}}$ |
| Dissolved oxygen | - 0.9260257 | $\mathbf{6.51 \times 10^{-10}}$ |
| DOC | - 0.6850793 | $\mathbf{4.35 \times 10^{-4}}$ |
| DIC | 0.8879038 | $\mathbf{3.53 \times 10^{-8}}$ |
| $NO_3^-$ | - 0.4288552 | **0.046** |
| $NH_x$ | 0.8445109 | $\mathbf{7.72 \times 10^{-7}}$ |
| Cu | -0.8852587 | $\mathbf{1.29 \times 10^{-4}}$ |
| Fe | 0.7125153 | $\mathbf{9.31 \times 10^{-3}}$ |
| ΣREEs | 0.9716254 | $\mathbf{1.38 \times 10^{-7}}$ |

Table S4. Results of the Kruskal-Wallis tests comparing abiotic parameters between sampling days across depths. DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; $NO_3^-$, nitrate; NHx, ammonium and ammonia.

| **Abiotic parameter** | **Depth (m)** | ***p*-value** |
|---|---|---|
| Temperature | 0 | 0.1017 |
| | 3 | 0.1017 |
| | 6 | 0.4966 |
| | 18 | 0.1653 |
| Dissolved oxygen | 0 | 0.1801 |
| | 3 | 0.1561 |
| | 6 | 1 |
| | 18 | 0.1905 |
| DOC | 0 | 0.1017 |
| | 3 | 0.123 |
| | 6 | 0.1653 |
| | 18 | 0.349 |
| DIC | 0 | 0.1801 |
| | 3 | 0.1561 |
| | 6 | 0.1653 |
| | 18 | 0.2062 |
| $NO_3^-$ | 0 | 0.08208 |
| | 3 | 0.3679 |
| | 6 | 0.7165 |
| | 18 | NA |

|  |  |  |
|---|---|---|
| $NH_x$ | 0 | 0.09072 |
|  | 3 | 0.1478 |
|  | 6 | 0.2231 |
|  | 18 | 0.2173 |

Table S5. Results of the Wilcoxon test comparing abiotic parameters between diel periods (day or night) across depths. DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; $NO_3^-$, nitrate; NHx, ammonium and ammonia.

| **Abiotic parameter** | **Depth (m)** | ***p*-value** |
|---|---|---|
| Temperature | 0 | 0.6625 |
|  | 3 | 0.6625 |
|  | 6 | 0.6579 |
|  | 18 | 0.6625 |
| Dissolved oxygen | 0 | 1 |
|  | 3 | 0.3827 |
|  | 6 | 0.08086 |
|  | 18 | 1 |
| DOC | 0 | 1 |
|  | 3 | 1 |
|  | 6 | 0.2683 |
|  | 18 | 0.8248 |
| DIC | 0 | 0.6625 |
|  | 3 | 0.6625 |
|  | 6 | 0.6625 |
|  | 18 | 0.5066 |
| $NO_3^-$ | 0 | 1 |
|  | 3 | 0.505 |
|  | 6 | 1 |
|  | 18 | NA |
| $NH_x$ | 0 | 1 |
|  | 3 | 0.5066 |
|  | 6 | 0.6625 |
|  | 18 | 1 |

Table S6. Results of the Kruskal–Wallis test comparing alpha diversity indices between depths for the Bacteria and Eukaryote domains. Significant values are in bold.

| **Domain** | **Alpha diversity** | ***p*-value** |
|---|---|---|
| Bacteria | Shannon index | **0.03197** |
|  | Observed richness | 0.1773 |
|  | Pielou’s evenness | 0.1032 |
| Eukaryote | Shannon index | 0.1632 |
|  | Observed richness | 0.4010 |
|  | Pielou’s evenness | 0.6783 |

Table S7. Results of Dunn's post-hoc test for pairwise comparisons of Shannon index between depths for the Bacteria domain following the Kruskal–Wallis test. *p*-values are adjusted using the Bonferroni correction. Significant values are in bold.

| **Comparison** | **Z-score** | **Unadjusted *p*-value** | **Adjusted *p*-value** |
|---|---|---|---|
| 0 m – 3 m | -0.04082483 | 0.967435545 | 1 |
| 0 m – 6 m | 0.97979590 | 0.327186878 | 1 |
| 0 m – 18 m | -1.91876697 | 0.055013832 | 0.33008299 |
| 3 m – 6 m | 1.02062073 | 0.307434166 | 1 |
| 3 m – 18 m | 1.87794214 | 0.060389081 | 0.36233449 |
| 6 m – 18 m | 2.89856286 | 0.003748772 | **0.02249263** |

Table S8. Variation in bacterial and eukaryote community composition explained by depth, sampling day, or diel period, tested using PERMANOVA. Significant values are in bold. SumOfSqs, sum of squares.

| | **Bacteria** | | | | | **Eukaryote** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Environmental variable** | **dF** | **SumOfSqs** | **R2** | **F** | **Pr (>F)** | **dF** | **SumOfSqs** | **R2** | **F** | **Pr (>F)** |
| Depth | 1 | 1.3822 | 0.18602 | 5.2258 | **0.001** | 1 | 0.9993 | 0.13609 | 2.9580 | **0.002** |
| Sampling day | 1 | 0.7963 | 0.10089 | 2.5331 | **0.008** | 1 | 0.9147 | 0.12457 | 2.7078 | **0.003** |
| Diel period | 1 | 0.0986 | 0.01249 | 0.3135 | 0.987 | 1 | 0.1410 | 0.01920 | 0.4173 | 0.979 |
| Depth : Sampling day | 1 | 0.3023 | 0.03829 | 0.9615 | 0.490 | 1 | 0.3567 | 0.04857 | 1.0558 | 0.405 |
| Depth : Diel period | 1 | 0.1012 | 0.01282 | 0.3220 | 0.992 | 1 | 0.1029 | 0.01402 | 0.3047 | 0.990 |
| Sampling day : Diel period | 1 | 0.1140 | 0.01444 | 0.3626 | 0.987 | 1 | 0.1583 | 0.02156 | 0.4687 | 0.959 |
| Depth : Sampling day : Diel period | 1 | 0.0688 | 0.00872 | 0. 2189 | 1 | 1 | 0.2784 | 0.03792 | 0.8242 | 0.683 |
| Residual | 16 | 5.0298 | 0.63724 | | | 13 | 4.3916 | 0.59807 | | |
| Total | 23 | 7.8931 | 1.00000 | | | 20 | 7.3429 | 1.00000 | | |

Table S9. ANOVA by contrast (999 permutations) on the Bray-Curtis dissimilarity index of each community. Significant values are in bold.

| | **Bacteria** | | | | **Eukaryote** | | | |
|---|---|---|---|---|---|---|---|---|
| **Abiotic factor** | **dF** | **SumOfSqs** | **F** | **Pr (>F)** | **dF** | **SumOfSqs** | **F** | **Pr (>F)** |
| Temperature | 1 | 1.5772 | 7.9847 | **0.001** | 1 | 1.2595 | 5.2399 | **0.001** |
| Dissolved oxygen | 1 | 0.6676 | 3.3796 | **0.001** | 1 | 0.5745 | 2.3900 | **0.007** |
| DOC | 1 | 0.2878 | 1.4570 | 0.134 | 1 | 0.4833 | 2.0107 | **0.028** |
| DIC | 1 | 0.3495 | 1.7692 | 0.066 | 1 | 0.4627 | 1.9251 | **0.028** |
| $NO_3^-$ | 1 | 0.2616 | 1.3242 | 0.187 | 1 | 0.2482 | 1.0328 | 0.397 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $NH_x$ | 1 | 0.4666 | 2.3622 | **0.010** | 1 | 0.3344 | 1.3912 | 0.142 |
| Residual | 17 | 3.3580 | | | 14 | 3.3650 | | |

Table S10. djusted $R^2$ values from variance partitioning of bacterial and eukaryotic community composition explained by selected abiotic factors.

| **Bacteria** | | **Eukaryote** | |
|---|---|---|---|
| **Abiotic factor** | **Adjusted $R^2$** | **Abiotic factor** | **Adjusted $R^2$** |
| Dissolved oxygen | 0.05712 | Dissolved oxygen | 0.00644 |
| Temperature | 0.05355 | Temperature | 0.04680 |
| $NH_x$ | 0.03543 | DIC + DOC | 0.07045 |

Table S11. ANOVA by contrast (999 permutations) on the Bray-Curtis dissimilarity index of each community, based on sample collected during the day. Significant values are in bold.

| **Abiotic factor** | **dF** | **SumOfSqs** | **F** | **Pr (>F)** |
|---|---|---|---|---|
| Temperature | 1 | 0.35611 | 2.6143 | **0.017** |
| Dissolved oxygen | 1 | 0.31167 | 2.2880 | **0.026** |
| DOC | 1 | 0.27813 | 2.0418 | 0.053 |
| DIC | 1 | 0.20185 | 1.4818 | 0.163 |
| $NO_3^-$ | 1 | 0.14284 | 1.0486 | 0.427 |
| $NH_x$ | 1 | 0.23201 | 1.7032 | 0.111 |
| Cu | 1 | 0.73261 | 5.3782 | **0.001** |
| Fe | 1 | 0.36525 | 2.6813 | **0.014** |
| ΣREEs | 1 | 0.49113 | 3.6054 | **0.008** |
| Residual | 2 | 0.27244 | | |

Table S12. Mean body length and density across sampling days, diel periods and depths.

| **Sampling day** | **Diel period** | **Depth (m)** | **Mean body length (mm)** | **Density (individuals/L)** |
|---|---|---|---|---|
| 1 | Night | 0 | 1.8975 | 0.9378 |
| 1 | Day | 0 | 1.9009 | 0.9586 |
| 1 | Night | 3 | 1.4144 | 1.1657 |
| 1 | Day | 3 | 1.4117 | 1.1582 |
| 1 | Night | 6 | 1.2228 | 0.1198 |
| 1 | Day | 6 | 1.2197 | 0.1594 |
| 2 | Night | 0 | 1.8613 | 0.7592 |
| 2 | Day | 0 | 1.8808 | 0.8529 |
| 2 | Night | 3 | 1.3888 | 1.1348 |
| 2 | Day | 3 | 1.3751 | 1.1224 |
| 2 | Night | 6 | 1.2482 | 0.1276 |
| 2 | Day | 6 | 1.2451 | 0.1176 |

Table S13. Results of the Kruskal–Wallis test comparing mean body length and density between depths. Significant values are in bold.

| **Biotic parameter** | ***p*-value** |
|---|---|
| Body length | **0.007277** |
| Density | **0.007277** |

Table S14. Results of the Wilcoxon test comparing mean body length and density between sampling days and diel periods (day or night) across depths.

| | | **Sampling days** | **Diel period** |
|---|---|---|---|
| **Biotic parameter** | **Depth (m)** | ***p*-value** | |
| Body length | 0 | 0.3333 | 0.6667 |
| | 3 | 0.3333 | 0.6667 |
| | 6 | 0.3333 | 0.6667 |
| Density | 0 | 0.3333 | 0.6667 |
| | 3 | 0.3333 | 0.6667 |
| | 6 | 0.6667 | 1 |

Table S15. Table of isotopic values for $\delta^{13}C$ and $\delta^{15}N$ measured in *Daphnia pulicaria* and particulate organic matter (POM).

| | | | ***Daphnia pulicaria*** | | **POM** | |
|---|---|---|---|---|---|---|
| **Sampling day** | **Diel period** | **Depth (m)** | **$\delta^{13}C$** | **$\delta^{15}N$** | **$\delta^{13}C$** | **$\delta^{15}N$** |
| 1 | Night | 0 | -42.90 | 2.97 | -33.78 | -0.43 |
| 1 | Day | 0 | -42.49 | 6.05 | -35.52 | 0.30 |
| 1 | Night | 3 | -45.11 | 1.82 | -44.39 | -2.03 |
| 1 | Day | 3 | -43.23 | 2.03 | -44.68 | -2.72 |
| 1 | Night | 6 | -43.67 | 2.45 | -43.06 | 1.13 |
| 1 | Day | 6 | -42.75 | -0.03 | -42.58 | 0.12 |
| 1 | Night | 18 | | | -51.51 | -1.12 |
| 1 | Day | 18 | | | -44.69 | -4.97 |
| 2 | Night | 0 | -35.91 | 2.01 | -34.51 | 2.56 |
| 2 | Day | 0 | -34.90 | 3.46 | -34.00 | 2.42 |
| 2 | Night | 3 | -43.62 | 2.17 | -41.48 | 0.96 |
| 2 | Day | 3 | -42.82 | 1.73 | -48.06 | 0.43 |
| 2 | Night | 6 | -41.04 | 1.96 | -41.10 | -1.25 |
| 2 | Day | 6 | -41.88 | 1.15 | -41.01 | -0.87 |
| 2 | Night | 18 | | | -42.77 | -1.30 |
| 2 | Day | 18 | | | -43.12 | -2.96 |
| 3 | Night | 0 | | | -36.61 | 1.32 |
| 3 | Day | 0 | | | -35.29 | 1.40 |
| 3 | Night | 3 | | | -39.33 | 1.50 |
| 3 | Day | 3 | | | -41.78 | 0.63 |
| 3 | Night | 6 | | | -40.45 | -0.29 |

| | | | | |
|---|---|---|---|---|
| 3 | Day | 6 | -38.15 | -0.06 |
| 3 | Night | 18 | -41.87 | -1.41 |
| 3 | Day | 18 | -42.59 | -2.57 |

Table S16. Results of the Kruskal–Wallis test comparing $\delta^{13}C$ and $\delta^{15}N$ values between depths for *Daphnia pulicaria* and the particulate organic matter (POM). Significant values are in bold.

| **Stable isotope** | **Organism** | ***p*-value** |
|---|---|---|
| $\delta^{13}C$ | *Daphnia pulicaria* | 0.09212 |
| | POM | **0.001145** |
| $\delta^{15}N$ | *Daphnia pulicaria* | 0.07748 |
| | POM | **0.0003174** |

Table S17. Results of Dunn's post-hoc test for pairwise comparisons of $\delta^{13}C$ and $\delta^{15}N$ values between depths for particulate organic matter following the Kruskal–Wallis test. *p*-values are adjusted using the Bonferroni correction. Significant values are in bold.

| **Stable isotope** | **Comparison** | **Z-score** | **Unadjusted *p*-value** | **Adjusted *p*-value** |
|---|---|---|---|---|
| $\delta^{13}C$ | 0 m – 3 m | 3.1026870 | 0.0019177234 | **0.011506341** |
| | 0 m – 6 m | 2.0004166 | 0.0454552948 | 0.272731769 |
| | 0 m – 18 m | 3.7150594 | 0.0002031559 | **0.001218936** |
| | 3 m – 6 m | -1.1022704 | 0.2703441407 | 1 |
| | 3 m – 18 m | -0.6123724 | 0.5402913746 | 1 |
| | 6 m – 18 m | -1.7146428 | 0.0864107330 | 0.518464398 |
| $\delta^{15}N$ | 0 m – 3 m | 0.6531973 | 0.5136291134 | 1 |
| | 0 m – 6 m | 2.4903146 | 0.0127630075 | 0.0765780452 |
| | 0 m – 18 m | 3.8783588 | 0.0001051636 | **0.0006309813** |
| | 3 m – 6 m | 1.8371173 | 0.0661925797 | 0.3971554783 |
| | 3 m – 18 m | -3.2251615 | 0.0012590163 | **0.0075540976** |
| | 6 m – 18 m | -1.3880442 | 0.1651235901 | 0.9907415408 |

Table S18. Results of the Wilcoxon test comparing $\delta^{13}C$ and $\delta^{15}N$ values between diel periods (night or day) per depth for *Daphnia pulicaria* and the particulate organic matter (POM).

| | ***Daphnia pulicaria*** | | **POM** | |
|---|---|---|---|---|
| | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{13}C$ | $\delta^{15}N$ |
| **Depth (m)** | ***p*-value** | | | |
| 0 | 0.6667 | 0.3333 | 1 | 1 |
| 3 | 0.3333 | 0.6667 | 0.2 | 0.4 |
| 6 | 1 | 0.3333 | 0.7 | 0.4 |
| 18 | | | 1 | 0.2 |

Table S19. Results of the Kruskal-Wallis test comparing $\delta^{13}C$ and $\delta^{15}N$ values between sampling days per depth for the particulate organic matter.

| | ***p*-value** | |
|---|---|---|
| **Depth (m)** | **$\delta^{13}C$** | **$\delta^{15}N$** |
| 0 | 0.3679 | 0.1017 |
| 3 | 0.3679 | 0.1561 |
| 6 | 0.1017 | 0.1561 |
| 18 | 0.1017 | 0.3679 |

Table S20. Results of the Wilcoxon test comparing $\delta^{13}C$ and $\delta^{15}N$ values between sampling days per depth for *Daphnia pulicaria*.

| | ***p*-value** | |
|---|---|---|
| **Depth (m)** | **$\delta^{13}C$** | **$\delta^{15}N$** |
| 0 | 0.3333 | 0.6667 |
| 3 | 0.6667 | 1 |
| 6 | 0.3333 | 1 |

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