University of Belgrade, Technical Faculty in Bor

30th International Conference Ecological Truth & Environmental Research 2023

Proceedings

Editor

Prof. Dr Snežana Šerbula

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PREFACE

The 30th international conference Ecological Truth & Environmental Research – EcoTER'23 kept three areas in focus: ecology, environmental protection and sustainable development. The conference will be held on Mt Stara Planina in hotel Stara Planina, Serbia, 20–23 June 2023. The monograph is published on the occasion of the 30th anniversary of the conference. On behalf of the scientific and organizing committee, it is a great honor and pleasure to wish all the participants a warm welcome to the conference.

The monograph is published on the occasion of the 30th anniversary of the conference.

We hope to convey the message of the conference, which is that a transformation of attitudes and behavior would bring the necessary changes. This is also an opportunity for the participants who are experts in this field to exchange their experiences, expertise and ideas, and also to consider the possibilities for their collaborative research.

The 30th international conference Ecological Truth & Environmental Research – EcoTER'23 is organized by the University of Belgrade, Technical Faculty in Bor, and co-organized by the University of Banja Luka, Faculty of Technology, the University of Montenegro, Faculty of Metallurgy and Technology – Podgorica, the University of Zagreb, Faculty of Metallurgy – Sisak, the University of Pristina, Faculty of Technical Sciences – Kosovska Mitrovica and the Association of Young Researchers, Bor.

These Proceedings 103 papers from the authors coming from the universities, research institutes and industries in 11 countries: Australia, USA, Brazil, Spain, Portugal, Libya, Italy, Bulgaria, Bosnia and Herzegovina, North Macedonia, and Serbia.

As a part of this year's conference, the 5th Student Session – EcoTERS'23 is being held. We appreciate the contribution of the students and their mentors who have also participated in the conference.

The support of the Gold donor and their willingness and ability to cooperate has been of great importance for the success of the EcoTER'23. The organizing committee would like to extend their appreciation and gratitude to the Gold donor of the conference for their donation and support.

We appreciate the effort of all the authors who have contributed to these Proceedings. We would also like to express our gratitude to the members of the scientific and organizing committees, reviewers, speakers, chairpersons and all the conference participants for their support to the EcoTER'23. Sincere thanks go to all the people who have contributed to the successful organization of the EcoTER'23.

Prof. Snežana Šerbula,

President of the scientific and organizing committee

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Student Section

ECOENZYMATIC STOICHIOMETRY AS AN EMERGING METHOD IN THE ASSESSMENT OF SOIL HEAVY METAL POLLUTION

Jelena Milosavljević[1*], Snežana Šerbula[1], Ana Radojević[1], Tanja Kalinović[1], Jelena Kalinović[1]

[1]University of Belgrade, Technical Faculty in Bor, V.J. 12, 19210 Bor, SERBIA

[*]*jmilosavljevic@tfbor.bg.ac.rs*

Abstract

Environmental pollution with heavy metals is one of the most important problems that should be addressed worldwide. Thus, efforts have been made in the development and evaluation of different soil pollution indicators. Among them, soil enzyme activity is regarded as the most prominent indicator, considering its enrolment in soil organic matter decomposition and the soil nutrient cycling. Special emphasis is given to the ecoenzymatic stoichiometry, which is regarded as an emerging method in soil pollution assessment. Based on the ratio of the enzyme activity, the ecoenzymatic stoichiometry can be used to assess the soil microbial metabolic limitation under various environmental disturbances. Taking into account different methods for ecological resorting of the contaminated environment, phyotremediation is considered as ecological and cost effective. Thus the evaluation of possible application of ecoenzymatic stoichiometry in the assessment of the impact of phytoremediation on soil structure and function is of great importance.

Keywords: heavy metals, soil pollution, soil enzyme activities, microbial limitations.

INTRODUCTION

Soil pollution with heavy metals has become a global problem due to their environmental persistence which led to degradation of soil quality and functions. Anthropogenic activities, such as mining, smelting, industrialization, and urbanization, are considered as the greatest sources of these non-biodegradable contaminants. Accumulation of heavy metals in soil could be harmful to human health through the soil-plant systems. Considering this, special emphasis is given to the selection of suitable indicators for soil heavy metal pollution assessment [1–3].

Soil extracellular enzymes secreted by plant and microbial cells, have a critical role in decomposition of the soil organic matter and the soil nutrient cycling [4–7]. Soil enzyme activities are regarded as powerful biological indicators due to the fact that they are one of the first soil properties that are altered under environmental disturbances. Thus, they are recognized as an early warning tool for the soil quality assessment, which integrate information about microbial status and soil physico-chemical properties [4,7].

Soil enzyme activity is related to both abiotic and biotic stressors [5]. The enzymes may respond differently to the environmental disturbances, so the use of different enzyme-based indicators may be required in the soil quality assessment [8]. The assessment of soil heavy metal pollution, using soil enzyme activities, include single enzyme index, combined enzyme index, enzyme-based functional diversity index, microbiological stress index, etc. [7]. Among the methods employing the enzymatic activity, ecoenzymatic stoichiometry (which is based on the ratio of the enzyme activity) is a promising methodology which can be used to assess the soil microbial metabolic limitation under various environmental managmements or disturbances [6,7]. Extracellular enzyme stoichiometry reveals nutrient requirements of microorganisms and environmental nutrients supply, whereby its evaluation facilitates the understanding of the soil nutrient circulation and balance [5]. Thus, this paper focuses on the application of ecoenzymatic stoichiometry, as an emerging method, in the environmental studies, especially for the assessment of heavy metal pollution after phytoremediation.

SOIL ENZYME ACTIVITY AND ECOENZYMATIC STOICHIOMETRY

Soil enzymes have a crucial role in nutrient biogeochemistry, whereby the nutrient cycling between plants and soil is mediated by microbes which secrete extracellular enzymes to degrade different organic compounds [9,10]. Thus, microbes provide plants with nutrients, however in the case of nutrient deficiency, microbes compete with roots for nutrients [10]. Taking into account that microbes have a competitive advantage compared to plants when it comes to acquiring nutrients, if microbes are limited by a certain soil nutrient, plants should also be limited [11].

By combining basics of biological, chemical and physical principles, ecological stoichiometry can be a useful tool for understanding the nutrient cycling in ecosystems. The central concept of ecological stoichiometry is stoichiometric homeostasis, which can be defined as the ability of organisms to maintain nutrient composition relatively constant, irrespective of changing nutrient status in the environment [12].

The activities of soil enzymes can reflect microbial nutrient demand, while the stoichiometric relationships between enzyme activities reflect the ability of microorganisms to use different resources (i.e. C, N, O, P and S) from environments [9]. As an emerging methodology, ecoenzymatic stoichiometry includes different parameters associated with enzyme activities into specific microbial characteristics (i.e. microbial metabolic limitations) [2]. Among soil enzymes, C-, N- and P-acquiring enzymes are of crucial importance since they have a significant contribution in energy flow and nutrient release [11].

The most-frequently analyzed soil enzyme activities linked to the microbial metabolism include [9,13]:

C-acquiring enzymes:

- β-glucosidase (BG) – catalyzes the terminal reaction in degradation of cellulose,
- β-1,4-N-acetylglucosaminidase (NAG) – catalyzes the terminal reaction in degradation of chitin;

N-acquiring enzymes:

- leucine aminopeptidase (LAP) – catalyzes the hydrolysis of leucine and other hydrophobic amino acids from the N-terminus of the polypeptides;

P-acquiring enzymes:

- acid or alkaline phosphatase (ACP or ALP) – catalyzes the hydrolysis of phosphate esters (i.e. phosphomonoesters, phosphodiesters, or phosphosaccharides) that release phosphate.

Specific activity of soil enzymes (i.e. activity g^{-1} soil organic matter) is used for the stoichiometric analyses [14]. Stoichiometric analyses of enzyme activity on the example of β-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), β-glucosidase (BG) and acid phosphatase (ACP) is conducted as [15]:

$$C{:}N_{EEA} = \ln BG/\ln (LAP + NAG) \tag{1}$$

$$C{:}P_{EEA} = \ln BG/\ln ACP \tag{2}$$

$$N{:}P_{EEA} = \ln (LAP + NAG)/\ln ACP \tag{3}$$

where $C{:}N_{EEA}$ represents the ecoenzymatic C to N ratio; $C{:}P_{EEA}$ the ecoenzymatic C to P ratio; and $N{:}P_{EEA}$ the ecoenzymatic N to P ratio.

Obtained ratios could reflect microbial C-limitations (or C-shortage) relative to N (increased BG: (LAP + NAG) ratio), C-limitations (or C-shortage) relative to P (increased BG:ACP ratio) or N-limitations (or N-shortage) relative to P (increased (LAP + NAG):ACP ratio) [13].

Microbial nutrient limitation analyzed by the vector analysis (length, L; angle, A) of the ecoenzymatic stoichiometry on the example of BG, NAG, LAP, ACP activities can be calculated with the equations (4) and (5) as suggested by Li *et al.* [15]:

$$\text{Vector L} = \sqrt{(\ln BG/\ln[NAG + LAP]^2 + (\ln BG/\ln ACP)^2} \tag{4}$$

$$\text{Vector A} = \text{Degrees}\ (\text{ATAN2}(\ln BG/\ln ACP), (\ln BG/\ln[NAG{+}LAP]))) \tag{5}$$

Vector L represents C limitation (longer vector L indicates greater C limitation); vector A denotes N and P limitation (vector A >45° indicates P limitation and <45° indicates N limitation) [15].

Sinsabaugh *et al.* [14] conducted a global-scale meta-analysis using data from 40 ecosystems, and found that, for hydrolases, the ratios of specific C, N, and P acquisition activities are close to 1:1:1. The C:N:P ratio of enzyme activities is influenced by different environmental factors, as well as anthropogenic disturbances [9].

ASSESSMENT OF SOIL HEAVY METAL POLLUTION BY ECOENZYMATIC STOICHIOMETRY

High concentrations of heavy metals in soil can be toxic to soil microbes since heavy metals can inhibit enzymes by reacting with the active catalytic sites (direct inhibition) or with groups involved in maintaining the enzyme structure (indirect inhibition). Thus, special emphasiz is given to understanding the relations between heavy metals, environmental factors and soil microbial activities [8]. Monitoring and assessment of soil heavy metal pollution can be performed by ecoenzymatic stoichiometry with a special focus on a nutrient cycling and microbial limitations in rhizospheric soil during phytoremediation [2,3].

Rhizosphere represents a hotspot where nutrient cycling among the soil, microbes and plants takes place. This cycling is mediated by enzymes that are produced by soil microorganisms and plant roots. It is estimated that up to 40% of photosynthetically fixed carbon is provided to microbes by plants, resulting in higher microbial activity in rhizospehere compared to the bulk soil. Also, the presence of plant roots affects soil nutrient cycling and C decomposition through altering soil physical properties and heavy metal availability by releasing protons and organic acids [3,15].

The ecoenzymatic stoichiometry can be used for determination of nutritional status and microbial nutrient limitations, or responses of microorganisms to the environmental changes. Cui *et al*. [16] denoted that enzyme stoichiometry method could be used to predict the response of soil microbial structure and element cycling to different long-term fertilization regimes. In the study by Zhang *et al*. [5] the effects of vegetation restoration on soil ecoenzymatic stoichiometry was evaluated. The vegetation and soil from one farmland and two restored land-use types were assessed, and the activity of β-glucosidase, β-1,4-*N*-acetylglocosaminidase, leucine aminopeptidase and alkaline phosphatase were determined. Soil ecoenzymatic C:N:P acquisition ratios deviated from the 1:1:1 ratio and was dependant on the nutrient availability and microbial nutrient demand. The results provided useful knowledge about nutrient cycling and microbial limitation in the restored ecosystems.

It should be pointed out that soil enzyme activities are regarded as sensitive indicators of soil microbial metabolism and nutrient cycling changes, which can be used to assess the impact of phytoremediation on soil structure and function [3].

Soil ecoenzymatic stoichiometry could be employed to study nutrient cycling and microbial limitation during phytoremediation. Duan *et al*. [3] performed a study to improve the assessment of ecological risks in soil polluted by heavy metals (Cu, Zn, Pb and Cd) and to give a theoretical basis for improvement of phytoremediation efficiency. The ecoenzymatic stoichiometry was used to examine the microbial limitation in soil near the copper mine. The activities of soil C- acquiring enzymes (β-glucosidase, cellobiohydrolase), N-acquiring enzymes (*N*-acetylglocosaminidase, leucine aminopeptidase), and P-acquiring enzyme (alkaline phosphatase) were analyzed in the rhizospheric and bulk soil of different plants (*Medicago sativa*, *Halogeton arachnoideus* and *Agropyron cristatum*). The vector analysis of soil enzyme activities showed that microbial C limitation was significantly less in the rhizospheric compared to the bulk soil, due to the root excretions and rhizodeposition. Also, rhizospheric soil was characterized with low P limitations. The higest ratios of N- and P-acquiring enzyme activities were observed in *Medicago sativa* (both rhizospheric and bulk soil) indicated low P limitation. The obtained results have shown that soil heavy metal content had negative effect on P limitation and positive effects on C limitations.

Wang *et al*. [2] stated that extracellular enzyme stoichiometry can successfully be used as a methodology for the soil heavy metal contamination assessment. Five-year phytoremediation experiment (*Lolium perenne* L., *Brassica napus* L., *Artemisia argyi*, *Silphium*, *Taraxacum*, *Populus*, and a control experiment) was performed in the vicinity of a smelter. The analysis included soil heavy metals content (Cu, Cd, Zn and Pb) and enzyme activities determination. The activities of enzymes involved in C (β-1,4-glucosidase, β-D-cellobiosidase), N (β-1,4-N-acetylglucosaminidase, L-leucine aminopeptidase) and P (alkaline phosphatase) cycling were further used for microbial nutrient limitation analysis. According to the enzymatic stoichiometry, *Lolium* treatment had the lowest microbial C limitation, which was indicative of the lowest heavy metal content. Soil heavy metal content (except Cu) was significantly negatively correlated ($p<0.05$) with microbial P limitation, whereby microbial C limitation was significantly positively correlated ($p<0.01$) with heavy metal content. Microbial C limitation was emphasized as a more appropriate indicator in assessing soil pollution compared to the P limitation.

Ecoenzymatic stoichiometry was emphasized as a promising methodology for heavy metal pollution evaluation in the study by Xu *et al*. [17], which was performed around Pb-Zn mine. The contents of Cd, Pb and Zn were determined in the soil, along with the activities of C-acquiring enzymes (β-1,4-glucosidase, β-D-cellobiosidase), N-acquiring enzymes (β-1,4-N-acetylglucosaminidase L-leucine aminopeptidase) and P-acquiring enzyme (alkaline phosphatase). By calculating the vector lengths and angles, microbial C and P limitation were quantified. Microbial C limitation was significantly positively correlated with Cd, Pb and Zn soil content, which was confirmed with decreasing trend of the vector length. Based on the obtained results, Xu *et al*. [17] stated that microbial metabolic limitation may be used as an index for evaluation of the effects of heavy metal pollution of soil.

The presence of heavy metals in the soil stimulates microbial C metabolism, which can be explained with the fact that microbes consume additional C sources to alleviate HMs toxic effects. On the other hand, heavy metals have negative effects on the growth and reproduction of microorganisms, thus reducing the limitation of P metabolism. Furthermore, the increased soil microbial C metabolism, due to heavy metal stress, affects the soil organic matter decomposition, thus releasing more available P from the organic matter. Also, metal ions (which are positively charged) can form complexes with the negatively charged functional groups (carboxyl and amino groups) on the surface of microorganisms, thus reducing microbial P demand [2,3].

CONCLUSION

Soil pollution with heavy metals has become a worldwide problem due to their environmental persistence and non-biodegradable nature. Bearing this in mind, efforts have been made for the development of indicators which could be used for monitoring and assessment of soil heavy metal pollution. Biological indicators which can be used for soil pollution monitoring, are of great importance since they respond rapidly to the environmental changes compared to the soil physico-chemical properties. The assessment of soil heavy metal pollution by soil biological properties could be performed by both single and combined enzyme indices. Ecoenzymatic stoichiometry, based on the soil enzyme activity, has been intensively studied as an indicator of the microbial nutrient demand and limitations. Microbial metabolic limitation was defined as a promising indicator for evaluation of soil heavy metal pollution, especially after phytoremediation.

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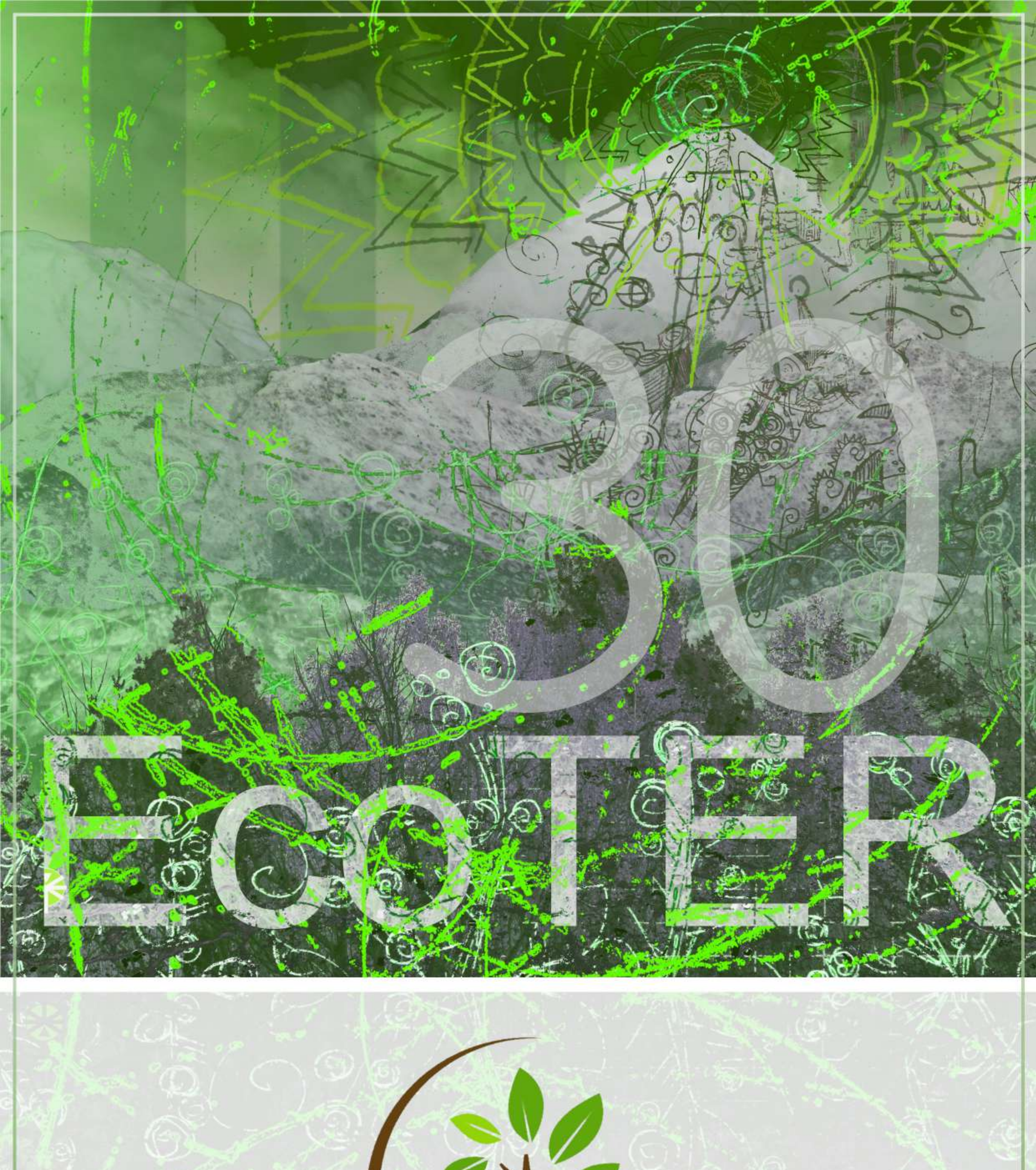

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