**1) Working title of your thesis.**

Glyphosate Accumulation in *Camassia Quamash* Bulbs of Coast Salish Prairies

**2) In 250 words or less, summarize the key background information needed to understand your research problem and question.**

*Camassia quamash* (camas) is a culturally and ecologically significant geophyte endemic to Coast Salish Prairies. The bulb is traditionally a staple source of carbohydrates for many Coast Salish tribes and has historically been traded across First Nations (Turner & Kuhnlein, 1983). Since the 1850s, Euro-American attempts at eradication of Indigenous peoples and lifeways have been detrimental to the reciprocal relationships by which the prairies and camas have thrived since time immemorial (Dunwiddie & Bakker, 2011; Turner & Kuhnlein, 1983). Opportunistic, non-native plant species have taken hold throughout the prairies and are at the forefront of restoration priorities (Dennehy et al., 2011). A key tool in reviving prairies and combatting non-native species is the use of herbicides (Dennehy et al., 2011; Wagner et al., 2017). Glyphosate, the most commonly used herbicide, may have health implications when consumed and perennial plants like camas are known to accumulate glyphosate in their tissues for years (Botten et al., 2021; van Bruggen et al., 2021; Wagner et al., 2017; Wood, 2019). Even with intensive management by restoration practitioners, non-native species will continue to be a restoration priority for the foreseeable future and herbicides will likely continue to be used (Dennehy et al., 2011). Understanding the degree to which camas bulbs accumulate glyphosate will inform traditional harvest safety and restoration practices (Wood, 2019).

**3) State your research question(s).**

How does the legacy of glyphosate application to a *Camassia quamash* habitat affect the accumulation of glyphosate in *Camassia quamash* bulbs?

**4) Situate your research problem within the relevant literature. What is the theoretical and/or practical framework of your research problem?**

Glyphosate is used in restoration and will continue to be used for the foreseeable future. The Coast Salish Prairie ecosystems are dwindling and at-risk for a number of reasons, with the pervasiveness of non-native opportunistic plants at the forefront (Dennehy et al., 2011; Dunwiddie & Bakker, 2011). Herbicides are considered a best-practice to control non-native species in Coast Salish Prairies and are used by both Indigenous and non-Indigenous land stewards, often combined with other restoration techniques (Dennehy et al., 2011). Due to their effectiveness, broad-applicability, and low-cost, glyphosate-based herbicides (GBHs) are the most commonly used herbicides in restoration and recommended for use in Coast Salish Prairies (Dennehy et al., 2011; Rivas-Garcia et al., 2022; Wagner et al., 2017). As the climate changes and globalization continues, new non-native species will take-hold (Willamette Partnership, 2020). Prairies may be particularly susceptible to non-native species because they are vastly open with little protection for air-borne seeds blown in from neighboring fields and traffic (Willamette Partnership, 2020). The heavy usage of herbicides will most likely continue as there is no end in sight to the need to manage non-native species (Dunwiddie & Bakker, 2011).

Glyphosate accumulates in perennial plants. Upon application to an ecosystem, plants will absorb glyphosate through leaves, stems, or roots (Kanissery et al., 2019; Wyrill & Burnside, 1976). The effect of the herbicide on the plant depends on the amount applied and plant resistance (Botten et al., 2021). Many plants survive exposure to sub-lethal amount of glyphosate and may store it for years (Botten et al., 2021; Wood, 2019). Young leaves, shoots, and roots accumulate glyphosate first, followed by older parts of the plants, and the compound continues to cycle through the plant until it is expelled as root exudates or contaminated tissues die (Wood, 2019; Wyrill & Burnside, 1976). Few glyphosate studies concern perennial plants or non-agricultural landscapes in temperate climates. Those that do show that glyphosate can accumulate in the roots of herbaceous perennials for at least 12 years and that herbaceous perennials are more prone to accumulation that woody perennials (Botten et al., 2021; Wood, 2019). Plant species and coldness of climate have be found to be the determining factors in glyphosate accumulation (Botten et al., 2021; Wood, 2019). Two perennial species with similar growth strategies but slightly different root structures accumulated glyphosate significantly differently (Wyrill & Burnside, 1976). Multiple studies have found that glyphosate accumulated in species less in ecosystems with colder winters than in ecosystems with warmer winters (Botten et al., 2021; Wood, 2019). The researchers speculated that this is because glyphosate may not be metabolized by bacteria while in the soil as quickly in colder climates as bacterial action is inhibited at low temperatures (Botten et al., 2021).

Consumption of glyphosate by humans at chronic, low levels may impact human health. With the substantial usage of glyphosate in agricultural systems throughout the world, studies concerning the health implications of glyphosate consumption have been abundant (Klingelhöfer et al., 2021). The pathway by which glyphosate kills plants, the shikimate pathway, does not exist in animals, leading to the thought that glyphosate consumption in low doses does not affect humans (Rivas-Garcia et al., 2022). Decades of research has generally supported this in short-term studies, but research within the past 6 years has brought new questions to light (Rivas-Garcia et al., 2022; van Bruggen et al., 2021). The shikimate pathway also exists in bacteria and the consumption of glyphosate may be affecting the human gut microbiome (van Bruggen et al., 2021). Tests done on rats have shown that chronic, below regulation doses of glyphosate disrupts the gut microbiome generationally and reduces immune responses (Buchenauer et al., 2022). Computer modelling studies analyzing the date from the Human Microbiome Project have found that nearly 70% of the bacteria found in the human gut microbiome has the shikimate pathway and would theoretically be sensitive to glyphosate (Mesnage & Antoniou, 2020). Leino et al. (2021) continued the microbiome analysis to find that 12-26% of bacteria in the human intestinal tract may be capable of being affected by glyphosate, while others have found this number to be 54% (Puigbò et al., 2022). Missing bacteria and bacterial community irregularities are linked to a host of diseases, syndromes, and neurological conditions (Vijay & Valdes, 2022)

**5) Explain the significance of this research problem. Why is this research important? What are the potential contributions of your work? How might your work advance scholarship?**

The habitat of *Camassia quamash* extends north to Vancouver, south to central California, and east into Montana and Utah. Much of this habitat has been suffering and is in active restoration, with herbicides, primarily GBHs, as a key tool. All Indigenous Nations that have camas in their historic territory, and Indigenous Nations beyond, traditionally consume camas to some extent. To understand the risks of consuming camas from sites that have been exposed to glyphosate, this thesis will assess land-use history of GBH use, application specifics of GBH use, soil composition, climate factors, and concentrations of glyphosate within camas bulbs. While the testing will confirm glyphosate concentrations in camas bulbs on site, the results will be able to be applied more broadly to other camas habitats. Analyzing the relationships between variables and concentrations will aid harvesters in determining safe areas to harvest and restoration practitioners in discerning the risks of when and where GBHs are used.

**6) Summarize your study design. If applicable, identify the key variables in your**   
**study. What is their relationship to each other? For example, which variables are you considering as independent (explanatory) and dependent (response)?**

To answer the question, “How does the legacy of glyphosate application to a *Camassia quamash* habitat affect the accumulation of glyphosate in the C *Camassia quamash* bulbs?” I propose an in vivo field study. There will be at least six field sites: three that have been sprayed with a GBH within one year and three that have not been exposed to GBHs. The field sites will have randomly selected sample plots. I would like to take a minimum of 30 samples sets: 3 from control sites and 7 from contaminated sites. More tests would be ideal.

The percentage of glyphosate in the GBH used, date of application, risk of air drift GBH exposure, GPS coordinates, and soil characteristics will be accounted for. Then, Camassia quamash bulbs will be collected from sample plots, transported to The Evergreen State College (TESC) campus lab, preserved, prepared for testing, and sent to an off-site laboratory. The off-site laboratory will test Camassia quamash samples for residue of glyphosate detectable to 0.01 mg/kg concentration.

I will also be noting climate and weather conditions since GBH application. Lower winter temperatures and the deep freezing of soil inhibit bacteria metabolization of glyphosate and may result in increased bioavailability (Botten et al., 2021; Wood, 2019). Since this data will be consistent across sites, they will not be a variable. The information will be collected from the National Oceanic and Atmospheric Administration database.

Null Hypothesis:

There is no detectable glyphosate in sampled Camassia quamash from contaminated and control sites.

Independent Variables:

* Date of sample
* Location
* Concentration of glyphosate in GBH applied to site
* Time since GBH application
* GBH application technique
* Risk of air drift GBH exposure
* Soil pH
* Soil organic matter content
* Soil structure

Independent variables were selected because they may affect the amount of glyphosate in the Camassia quamash bulbs sampled. According to the literature, the variables listed can affect the amount of glyphosate that is bioavailable in the soil (Kanissery et al., 2019). Concentration of glyphosate in GBH and application technique affects the quantity of glyphosate that is possibly bioavailable (Kanissery et al., 2019). The date(s) of GBH application affects glyphosate accumulation in soil (Ojelade et al., 2022) . Risk of air drift of GBH will inform the risk of any extra glyphosate being in the ecosystem (Olszyk et al., 2013). Glyphosate is immobilized in soils with higher organic matter content, higher clay content, and lower pH (Ojelade et al., 2022). Soils with these characteristics hold glyphosate for longer and may affect the bioavailability of glyphosate either negatively or positively (Ojelade et al., 2022).

There have been only a few studies measuring glyphosate concentrations in perennial plants in a temperate climate. The results showed that accumulation of glyphosate in plants is primarily affected by GBH application history, winter temperatures, and plant species (Botten et al., 2021; Wood, 2019). However, due to the lack of studies on this topic, accounting for more variables may inform future research and restoration practices.

Dependent Variable:

* Glyphosate concentration in Camassia quamash bulb samples.

Site Selection:

At least three control sites and three contaminated sites will be selected. The control site’s ideal conditions include (in order of priority) no known history of GBH use; at least 300 feet away from other areas that have been broadcast sprayed with GBHs; and current or future use as an Indigenous traditional harvest site. The contaminated site’s ideal conditions include (in order of priority) a dependable record of GBH usage with glyphosate amount, timing of application, and boom-sprayed application technique; at least 300 feet away from other areas that have been broadcast sprayed with GBHs; and current or future use as an Indigenous traditional harvest site.

If possible, there will be multiple control and multiple contaminated sites. Contaminated sites with different lengths of time since last GBH application and/or with confirmed repeated application and timing would be optimal.

Example case studies:

Wood, L. J. (2019). The presence of glyphosate in forest plants with different life strategies one year after application. *Canadian Journal of Forest Research*, *49*(6), 586–594. <https://doi.org/10.1139/cjfr-2018-0331>

Botten, N., Wood, L. J., & Werner, J. R. (2021). Glyphosate remains in forest plant tissues for a decade or more. *Forest Ecology and Management*, *493*, 119259. <https://doi.org/10.1016/j.foreco.2021.119259>

**7) Describe the data that will be the foundation of your thesis. Will you use existing**   
**data, or gather new data (or both)? Describe the process of acquiring or collecting**   
**data.**

I will be collecting my own data for this thesis. Data will be collected through personal communication with land management organizations, in-field sampling and laboratory testing, GIS land-use analysis, and historic climate reports.

*Sampling Methods:*

GBH use legacy data will be determined by personally communicating with land management organizations and through GIS land-use analysis.

Location data will be collected with personal cell phone location data, using an accuracy of 5 feet.

Soil samples will be taken in-field with a soil core sampler. Samples will then be brought back to The Evergreen State College laboratory to be analyzed on location.

*Camassia quamash* bulbs will be dug up from sample plots with as little disturbance as possible. Bulbs of traditional harvest size will be prioritized. Bulbs will then be brought back to TESC laboratory, cleaned, combined, and frozen. Once all samples are collected and processed, samples will be sent to an off-site laboratory to be tested for glyphosate using Liquid Chromatography Tandem-Mass Spectrometry. The laboratory has not yet been selected. Amounts of bulbs sampled and weight of samples will be determined in accordance with off-site laboratory specifications.

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Measurement** | **Cont/Cat** | **Method** |
| Concentration of glyphosate in GBH applied to site | % | Cont | Personal communication |
| Time since GBH application | Days, weeks, months | Cont | Personal communication |
| Risk of air drift GBH exposure | Yes/no | Cat | GIS land-use analysis using ArcPro |
| Location | GPS coordinates: decimal degrees | Cont | Personal cellphone: Android Galaxy (accuracy within 5 feet)  \*these may not be published if sensitive information |
| Soil pH | Digit on pH scale | Cont | pH meter, sample taken in field and transported to TESC lab |
| Soil organic matter content | % | Cont | Loss on ignition, sample taken in field and transported to TESC lab |
| Soil structure | Clay, sand, loam, etc | Cat | Visual observation |
| Date of sample | dd/mm/year | Cont | Calendar |
| Glyphosate concentration in Camassia quamash bulbs | mg/kg or ppm | Cont | Liquid Chromatography Tandem-Mass Spectrometry |

**8) Summarize your methods of data analysis. If applicable, discuss any specific**   
**techniques, tests, or approaches that you will use to answer your research question.**

Data will be entered into an Excel spreadsheet and analyzed in R with a 95% confidence interval. The dependent variable will be tested for normality with Shapiro-Wilk. If normality is found, then multilinear regression analyses will be performed for all continuous independent variables against the dependent variable. In addition, a check for collinearity will be performed and the multilinear regression analyses will be modified accordingly. If necessary, data will be log transformed.

For categorical independent variable analysis, I will use t-tests against the dependent variable.

**9) Address the ethical issues raised by your thesis work. Include issues such as risks to anyone involved in the research, as well as specific people or groups that might benefit from or be harmed by your thesis work, perhaps depending on your results. List any specific reviews you must complete first (e.g., Human Subjects Review or Animal Use Protocol Form).**

In my thesis process, it is likely that I will be communicating with the Confederated Tribes of the Chehalis Reservation and sampling on their ancestral Tribal lands. I need to be conscious of not inserting myself or making assumptions. There is risk of publishing information that Tribal members do not want published, damaging cultural resources and landscapes, continuing the harmful legacy of Western Science, and writing inaccurately in my final thesis publication.

I will be taking in-field soil and plant samples. There is a very minimal risk of having a negative effect on plant populations, soil structure, and the ecology of the ecosystem through sampling disturbance.

There is risk to myself in travel, in field with sampling tools, and in laboratory with equipment and methods.

**10) List specific research permits or permissions you need to obtain before you begin collecting data (e.g. landowner permissions, agency permits).**

I have not yet chosen specific sample sites because I have not begun my GIS land-use analysis. I will be researching Coast Salish Prairie wildlife preserves, conservation lands, and Confederated Tribes of the Chehalis Reservation lands. For wildlife preserves and conservation lands such as Glacial Heritage Preserve and Wolf Haven, I will contact land management organizations. For The Confederated Tribes of the Chehalis Reservation lands I will ask for permission from the Tribe. My contact there is William Thoms, the Tribe’s Cultural Resource Specialist. I have begun this conversation and he offered to give me access to Tribal lands to sample on. There will likely be more to this permissions than communication with William Thoms and I would like to discuss what information I collect can be published, such as GPS data, and in field sampling methods. I will consult with my thesis reader in these communication processes as well.

**11) Reflect on how your positionality as a researcher could affect your results and how you will account for this in the research process.**

I am an Anglo-American, cis-woman who grew up in an affluent family with college-educated parents. My heritage is murky. My understanding is that much of my mother’s side and my father’s side of the family left England and arrived in what is now known as the United States in the 1600s. If this is true, it is likely that my ancestors committed severe actions, possibly physical violence, against Indigenous peoples. Regardless, my farmer ancestors have thrived and profited off the land taken from Indigenous peoples by Euro-Americans. In addition, my grandmother has told me that her grandmother (my great-great-grandmother) was full-blooded Cherokee. A picture of her in traditional clothing seems to confirm this, but she is not listed on The Dawes Rolls. I don’t often speak of this potential Cherokee connection as it is not confirmed and I did not grow up practicing Cherokee traditions. However, both the early colonizer and potential Cherokee heritage affect my positionality as a researcher.

In my research, I am actively pursuing working with Indigenous First Foods, land stewardship, and First Nations. This line of work has been an interest of mine since a young age, before I knew of my heritage. Still, my research interests are partially informed by guilt, though they are mostly based in interest, respect, and a belief that reciprocal relationships with the land is the only way forward.

Additionally, I recognize that I tend to want my research to have a certain, unlikely outcome: that glyphosate does not accumulate in camas bulbs. I want to not have to substantially adjust restoration practices and I want all land to be safe to harvest from.

To account for my positionality, I will be thoughtful in how I communicate with Indigenous peoples throughout my thesis process. In my thesis writing, I will research ways that other Anglo-American scientists have addressed a context of genocide, Indigenous Knowledge, and Indigenous lifeways in their writing. I will adjust my writing accordingly and seek feedback from Indigenous peoples and non-Indigenous peoples with expertise in similar writing, potentially offering a monetary exchange. When I analyze results, I will remain curious throughout the process and work towards detaching myself from an outcome. Once my thesis is completed, I will give my results to Tribal Nations that I have worked with and step back. My job is to collect and analyze data, not to make a decision about safety of harvest for other people.

**12) Provide at least a rough estimate of the costs associated with conducting your**   
**research, if any. Provide details about each budget item so that the breakdown of the final cost is clear.**

Glyphosate testing expense is for Medallion Labs (*Medallion Labs*, n.d.). Medallion Labs is owned by General Mills LLC. This is the only laboratory that I could find that did not require the client to contact them for a quote. I will explore costs and dependability of other laboratories such as Synergistic Pesticide Lab (Portland, OR), The Great Plains Laboratory (Overland Park, KS), EnviroScience Inc (various locations in US), and Anresco Laboratory (San Fransisco, CA).

|  |  |  |  |
| --- | --- | --- | --- |
| **Expense** | **Cost Per Unit** | **Notes** | **Estimated Total Expense** |
| Travel | $0.625 / mile | Travel to and from sample sites  Travel to/from Glacial Heritage Preserve: 46 miles  Travel to/from Wolf Haven Prairie: 36 miles  Travel to/from Chehalis Tribal Center: 64 miles  Estimated 4 trips to/from sample sites maximum miles: 256 miles | $160 |
| Soil sampler | $0 | Borrow from TESC | $0 |
| Soil sampling tools | $0 | Borrow from TESC: pH meter, loss on ignition lab supplies | $0 |
| Sample storage: freezer bags | $11 | Storage for soil samples and bulbs: freezer bags, 60 pack | $11 |
| Camassia quamash bulb mail | $30 | Flat rate shipping | $30 |
| Glyphosate testing | $288/test | Cost at Medallion Labs for 30 tests | $8640 |
| Feedback compensation | $100/person | Ideally requesting feedback from 2-3 people (2 Indigenous; 1 non-Indigenous) | $300 |
| Thesis extension | $500/quarter | 1 quarter | $500 |
| Total: |  |  | $9641 |

**13) Provide a detailed working outline of your thesis.**

* Abstract
* Positionality Statement? (I would like to insert something to the effect of a positionality statement and am not sure where to put it – it would be quite different, less candid, than my statement in this document)
* Table of Contents
* List of Figures
* List of Tables
* Acknowledgements

1. Introduction
   1. Context and Roadmap
   2. Indigenous Lifeways and Well-Being
      1. Introduction
   3. Camassia Quamash
      1. Introduction
      2. Phylogeny
      3. Extent/Range
      4. Botany (description)
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   4. Coast Salish Prairie Restoration
      1. Introduction
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      3. Ecosystem Vulnerability
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   5. Glyphosate
      1. Introduction
      2. Use in Restoration
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      1. Introduction
      2. Controversy and Independence of Studies
      3. Regulations
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2. Glyphosate Accumulation in Camassia Quamash
   1. Introduction
   2. Methods
   3. Results
   4. Discussion
3. Conclusion

* References
* Appendix

**14) Provide a specific work plan and a timeline for each of the major tasks in the work plan. Be as realistic and specific as you can at this point, including the deadlines for Spring quarter.**

|  |  |
| --- | --- |
| Month | Items |
| December | * Thesis poster presentation * Submit Final Theis Prospectus * Set up notification alerts for relevant articles and publications |
| January | * Request glyphosate testing quotes from labs (Early January) * Apply for funding: MES Thesis Grants (Due January 13th) * Research funding potential * Continue Literature Review * Communicate with Thesis Reader for specific meeting schedule * Research site locations; communicate with appropriate agencies and nations * GIS analysis of land-use at and surrounding potential sampling sites * Request laboratory access from John Kirkpatrick |
| February | * Place Literature Review components within thesis outline and assess what needs to be added * Confirm sampling locations * GIS analysis of land-use at and surrounding sampling sites * Confirm laboratory methods * Work on rough draft of methods section of thesis * Work on rough draft of introduction section * Apply for funding: Washington Native Plant Society CPS (March 1st) |
| March | * Complete rough draft of entire Introduction section |
| April | * Request thesis extension (Due May 1st) * Work on rough draft of Chapter 2 |
| May | * Work on rough draft of Chapter 2 * Collect samples towards the end of May * Complete sections of thesis to extent possible * Process samples * Send samples to lab |
| June | * Complete soil testing in lab * Complete Methods section of thesis * Receive off-site laboratory results * Enter data into spreadsheet * Analyze results * Create figures and tables * Rough drafts of Results and Discussion sections of thesis * Rough draft of Conclusion * Final rough draft of thesis |
| July | * Analyze results * Request to present (Mid-July) * Final thesis due (August 1st at latest) |
| August | * Prepare thesis presentation * Thesis presentation |
| September | * Thesis presentation |

**15) Who (if anyone), beyond your MES thesis reader, will support your thesis (in or**   
**outside of Evergreen)? Be specific about who they are and in what capacity they will**   
**support your thesis. If you are working with an outside agency or expert, be specific**   
**about their expectations for your data analysis or publication of results.**

At this time, there will be no one else supporting my thesis outside of my thesis reader.

**16) Provide the 5 most important references you have used to identify the specific**   
**questions and context of your topic, help with issues of research design and analysis, and/or provide a basis for interpretation. Annotate these references with notes on how they relate to/will be helpful for your thesis. For any other sources cited in your prospectus in other answers, provide a complete bibliographic citation here as well.**

*Camas (Camassia spp) and riceroot (Fritillaria spp.): Two liliaceous “root” foods of the NW Coast Indians* (Turner & Kuhnlein, 1983)

Turner and Kuhnlein provide an ethnobotanical account of Coast Salish relationship with camas. They use first hand accounts, interviews, generational knowledge, and historic records. This resource will support the ethnobotanical context of my research and the significance of my research question.

*Glyphosate: Its Environmental Persistence and Impact on Crop Health and Nutrition* (Kanissery et al., 2019)

Kanissery et al., is a digestible introduction into the mechanisms of glyphosate and its persistence in the environment. The article aids in gaining a comprehensive understanding of glyphosate, GBHs, and AMPA. I will use it to inform background information and environmentally significant variables that need to be accounted for in my research.

*Glyphosate remains in forest plant tissues for a decade or more* (Botten et al., 2021)

To my knowledge, this is the most comprehensive study of the legacy of GBH use and subsequent accumulation of glyphosate in plants. The authors test two key components that are relevant to my research: herbaceous perennial plants and glyphosate usage over long periods of time. The research is not directly applicable because the study sites were in temperate forests, not prairies, and the herbaceous plants studied did not have bulbs. However, it uses relevant methods and analysis that I can apply to my experimental design. Their results also provide support for significance of my research question.

*Indirect Effects of the Herbicide Glyphosate on Plant, Animal, and Human Health Through its Effects on Microbial Communities* (van Bruggen et al., 2021)

This article supports the significance of my research question. While the focus on glyphosate, microbial communities, and health implications is fairly new, research presented in this article suggests that glyphosate may be severely effecting gut microbiomes. It is a comprehensive review of literature pre-2021 and has led me to newer articles of significance.

*Studies of the life history of Camassia quamash (Pursh)* Greene (Maclay, 1928)

This resource is a thesis written in 1928 at Washington State University. Maclay is consistently cited in camas research as much of the written botanical documentation on *Camassia quamash* is attributed to her. She gives a detailed biological account of *Camassia quamash*, including observations of growth patterns and life strategies that may be key to how a plant accumulates glyphosate.

Botten, N., Wood, L. J., & Werner, J. R. (2021). Glyphosate remains in forest plant tissues for a decade or more. *Forest Ecology and Management*, *493*, 119259. https://doi.org/10.1016/j.foreco.2021.119259

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