Rol ecosistémico de la zona riparia en sistemas dulceacuícolas en un escenario de cambio global

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PEDECIBA

UNIVERSIDAD DE LA REPÚBLICA URUGUAY

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PEDECIBA

ANII AGENCIA NACIONAL DE INVESTIGACIÓN E INNOVACIÓN

CSIC COMISIÓN SECTORIAL DE INVESTIGACIÓN CIENTÍFICA

Tesis presentada en el Programa de Desarrollo de las Ciencias Básicas (PEDECIBA) como requisito para la obtención del título de Doctora en Ciencias Biológicas.

Las actividades fueron llevadas a cabo en el Departamento de Ecología y Gestión Ambiental, Centro Universitario Regional del Este (CURE, Maldonado), Universidad de la República (UdelaR) bajo la orientación de la Dra. Mariana Meerhoff Scaffo y el Dr. Kirk O. Winemiller.

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Maldonado, 2022

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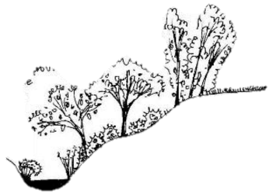

AGRADECIMIENTOS

Les agradezco en primer lugar a mis tutores, Dra. Mariana Meerhoff y Dr. Kirk Winemiller por sus invaluables aportes científicos, y su guía y apoyo durante este largo camino. Muy especialmente a Mariana, una inspiración para todas las nuevas generaciones de mujeres en la ciencia, siempre con la palabra justa y la humanidad tan necesaria para este proceso. ¡Gracias por tanto Mari!

A los miembros del tribunal, Dr. Luis Aubriot, Dra. Christine Lucas y Dra. Claudia Feijoó por aceptar ser parte y tomarse el tiempo para enriquecer este trabajo con sus comentarios e ideas. A los miembros de la Comisión de Seguimiento, Dr. Alejandro Brazeiro, Dra. Lorena Rodríguez-Gallego y Dra. Christine Lucas por sus siempre interesantes contribuciones y grandes mejoras al proyecto.

Al gran equipo del Convenio Paso Severino, Lorena Rodríguez-Gallego, Mariana Meerhoff, Gastón de León, Andrés Castagna, Soledad Costa, Leticia González y Lucia Cabrera con quienes compartimos innumerables salidas, reuniones, talleres e informes, y aportaron sin duda conocimiento variado y muy valioso a mi formación. A Lorena en particular por todas las charlas interesantes sobre ciencia y haber sido una excelente líder en esta nueva experiencia.

Al querido grupo del CURE, Franco, Guille, JP, Gige, Jpi, Kq, Iván, Mari, Clau, Ani, Mai, Marge, Lu C., Maite, Sami, Fran, Pauli, Lu. G., Canario, Pache, Bárbara, Sole, Ale, Miriam y muchos que deben estar faltando. Todos ustedes hacen los días de oficina y laboratorio disfrutables y divertidos, sobre todo las charlas científicas y de la vida tomando café. Que nunca falten! Especialmente a Lu, mi gran compañera de salidas de campo y largas horas de charla en la ruta, a Marge y Mai por su amistad incondicional, y a Ani por estar en todas.

Al Dr. Erik Jeppesen, la Dra. Annette Baattrup-Pedersen, y todo el excelente grupo que conocí en Silkeborg dentro y fuera del instituto. Muy especialmente a Juan Pablo y Celina por recibirme en Dinamarca; gracias a ellos tuve una estadía inolvidable. Tengo los mejores recuerdos de los veranos de trabajo, idas al lago, juntadas, conciertos y paseos. Gracias!

Al grupo de PONDERFUL, Coti, Lea, Emi, Bárbara, Marge, Flor, Maite, Franco y Mari con quienes hemos trabajado el último año e hicieron los muchos desafíos y días de campo del proyecto enormemente disfrutables.

A PEDECIBA, ANII y CSIC por el apoyo durante todo el proceso, gracias por la confianza y las oportunidades brindadas.

A los amigos de toda la vida de Maldonado y Montevideo por siempre preguntar y ponerse felices de verme disfrutar mi carrera, y estar cerca en los momentos complicados. Son fundamentales,

infinitas gracias! A mis amigas biólogas del comienzo de la carrera por compartir innumerables charlas y seguir acompañándonos en todas a pesar de la distancia: Pia, Sabri, Ana, Flo y Mari. Y a todos los nuevos amigos internacionales!

A mi familia por siempre estar incondicionalmente y creer en mí, y a la de Mateo (que hace mucho ya siento como propia) por hacerme sentir una más. Los adoro! Cada reencuentro con ustedes es de las cosas más lindas que nos pasan. Y sobre todo gracias por los sobrinos nuevos de estos años: Lorenzo, Gerónimo y Candelaria, y los que se seguirán sumando :)

Por último, a Mateo, mi persona favorita en el mundo. Además de las muchísimas cosas que pasaron en nuestros doctorados, pienso en todo lo vivido juntos en este tiempo y me parece increíble! Los viajes, lugares, y experiencias han hecho que sea una etapa espectacular, y no puedo esperar por todas las cosas nuevas por venir. Gracias por siempre impulsarme a más. Sin duda la vida es mucho más linda contigo.

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Artículo 2	**Calvo C.**, de León G., González-Carreira L., Costa S., Cabrera-Lamanna L., Castagna A., Meerhoff M. & Rodríguez-Gallego L. Methodology for subsurface monitoring of nutrient inputs to water systems. 2020. *ALHSUD*, 3, 7-9.
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Artículo 8 Pacheco J. P., **Calvo C.**, Aznarez C., Barrios-Gómez M., Meerhoff M., Jeppesen E. & Baattrup-Pedersen A. Periphyton biomass and life-form responses to a gradient of discharge under contrasting light and nutrients scenarios in experimental lowland streams. 2021. *Science of the Total Environment*, 806, 150505.

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RESUMEN

Las zonas buffer o riparias son las interfaces entre ecosistemas acuáticos y terrestres, y a pesar de la relativamente pequeña área que ocupan en la cuenca, promueven varios de los procesos que más resiliencia aportan a los ecosistemas acuáticos. Por la amplia gama de contribuciones positivas desde un punto de vista ambiental, su conservación y/o recuperación es una de las estrategias de restauración más frecuente en cuencas hidrográficas con calidad del agua degradada. En particular, esta estrategia se utiliza globalmente como una buena práctica de gestión para la reducción de la carga externa de nutrientes que alcanzan las aguas superficiales. Además, las zonas riparias influyen sobre las dinámicas hidrológicas y la cantidad de luz que llega a los ecosistemas acuáticos. Por ello, el ensamble de especies y varios atributos de las comunidades acuáticas dependen, entre otros factores, de la cobertura vegetal de la zona litoral. En el escenario actual de cambio climático e intensificación agrícola, se espera que varios procesos físicos, químicos y biológicos en la interfase tierra-agua sean afectados, con el aumento de la transferencia de nutrientes a los cuerpos de agua superficiales como una de las consecuencias más relevantes. Debido a su posición topográfica en el paisaje, las zonas riparias están generalmente muy expuestas a eventos climáticos extremos, como inundaciones y sequías, que pueden modificar sus dinámicas naturales. Además, pueden influir sobre la vulnerabilidad de los cuerpos de agua dulce al cambio climático, por su potencial rol mitigando los efectos del aumento de temperatura y de la ocurrencia de eventos extremos de precipitación.

El objetivo de este doctorado fue determinar el rol de las zonas riparias sobre la calidad del agua dulce superficial, desentrañando algunos factores de la interacción entre el cambio climático y cambios en los usos de la tierra en las cuencas de drenaje. Se buscó conectar patrones en campo a escala de cuenca, con análisis experimental de procesos del suelo, y análisis experimentales de las posibles respuestas dentro del cuerpo de agua a cambios indirectos potencialmente generados por la vegetación riparia. Para ello se combinó una revisión bibliográfica de estudios generados a nivel nacional en relación directa o indirecta a las zonas buffer, con muestreos intensivos en campo, y experimentos en mesocosmos. Se analizaron posibles efectos del cambio climático y del uso de la tierra sobre la retención de nutrientes, principalmente fósforo, por parte de la vegetación riparia (comparando pastizal, arbustal y bosque nativo) sobre el Embalse de Paso Severino (Florida, Uruguay). En un experimento anexo utilizando suelo de cultivo y bosque nativo del mismo sitio, simulamos en laboratorio escenarios de calentamiento y precipitaciones extremas para evaluar la liberación de nutrientes y cambios en los flujos de carbono. Asimismo, se analizaron experimentalmente las respuestas de un productor primario acuático (perifiton, en aguas corrientes) frente a factores estrechamente condicionados por la presencia de vegetación riparia: el ingreso de nutrientes y la disponibilidad de luz, en escenarios de pulsos de flujo.

En la revisión encontramos diversas líneas de investigación en conexión con la vegetación riparia, con la temática en general siendo aún incipiente y poco desarrollada a nivel nacional, pero creciendo de manera rápida. En el trabajo de campo, encontramos grandes cantidades de fósforo (la mayor parte en forma disuelta) movilizándose desde cultivos al Embalse de Paso Severino a través del agua de escorrentía superficial y subsuperficial, y a las zonas buffer desempeñando un papel fundamental en la retención de las mismas. Según nuestros resultados, una combinación de vegetación herbácea y leñosa alcanzaría la tasa máxima de retención de nutrientes, cuando se considera al fósforo y nitrógeno de manera simultánea. Encontramos evidencia de que la capacidad de amortiguamiento y consecuente beneficio directo para la disminución de la carga

externa de nutrientes podrían verse reducidos en escenarios climáticos futuros, ya que el transporte de nutrientes aumenta con el aumento en la tasa de escorrentía provocada por lluvias más intensas. En el marco de este componente desarrollamos metodologías innovadoras para la toma de muestras de agua subsuperficial.

Experimentalmente, encontramos evidencia de que el calentamiento intensifica la liberación de nutrientes del suelo agrícola. Con respecto al uso del suelo, en un escenario de anegamiento del suelo, encontramos que la liberación de fosfato fue sustancialmente mayor en el suelo de cultivo que en el bosque. Sin embargo, nuestra evidencia mostró que, en eventos de precipitación de mayor magnitud en los que el suelo efectivamente se inunda, los beneficios de la zona buffer no resultaron tan evidentes, ya que la liberación de fósforo al agua del bosque alcanzó valores similares al cultivo y el nitrógeno generalmente fue más alto en el bosque. Esto se vuelve relevante si la carga de nutrientes llegando desde la cuenca de captación al área buffer es alta y hay acumulación de nutrientes, comprometiendo el ciclado de los mismos. Registramos un cambio de sumidero a fuente de CO_2 en suelo de bosque bajo condiciones de calentamiento y disponibilidad de agua, sugiriendo que el calentamiento global podría acelerar la descomposición del carbono orgánico del suelo y, potencialmente, generará una retroalimentación positiva sobre el aumento de temperatura.

En el experimento en arroyos artificiales demostramos que los pulsos de flujo pueden tener efectos no lineales sobre el perifiton, tanto en términos de biomasa, como era de esperar, pero también que los efectos sobre la composición comunitaria. Estos efectos variaron fuertemente dependiendo de la estructura de la comunidad inicial (mediada por el flujo basal, la disponibilidad de luz y nutrientes). En un escenario de aumento de luz, nuestros resultados sugieren un mayor crecimiento del perifiton y una mayor resistencia contra el control de su biomasa ejercido naturalmente por los pulsos de flujo.

A lo largo de la tesis se encontró evidencia sobre la importancia de mantener o reestablecer a nivel local zonas de amortiguamiento para reducir la exportación de nutrientes desde suelos agrícolas. El rol de las zonas buffer va a ser especialmente relevante en escenarios futuros de aumento de temperatura y precipitación, aunque la efectividad de sus funciones puede verse también comprometida. Esta información es necesaria para generar o ajustar regulaciones, basadas en conocimiento científico, que potencien la resistencia y resiliencia de los ecosistemas acuáticos frente a diferentes perturbaciones externas como el ingreso de nutrientes o el cambio climático. Puntualmente, el establecimiento de vegetación riparia en la zona de fluctuación del nivel del agua sería un método efectivo para reducir la entrada de nutrientes al Embalse estudiado.

ABSTRACT

Buffer or riparian zones are the interfaces between aquatic and terrestrial ecosystems, and despite the relatively small area they occupy in the basin, they promote several of the processes that contribute the most resilience to aquatic ecosystems. Due to the wide range of positive contributions from an environmental point of view, its conservation and/or recovery is one of the most frequent restoration strategies applied in basins with degraded water quality. In particular, this strategy is used globally as a good management practice for reducing the external load of nutrients reaching surface waters. In addition, riparian zones influence hydrological dynamics and the amount of light that reaches aquatic ecosystems. For this reason, the set of species and various attributes of the aquatic communities depend, among other factors, on the vegetation cover of the margins. In the current scenario of climate change and agricultural intensification, several physical, chemical and biological processes at the land-water interface are expected to be affected, with increased transfer of nutrients to surface water bodies as one of the most important consequences. Due to their topographical position in the landscape, riparian zones are generally highly exposed to extreme climatic events, such as floods and droughts, which can modify their natural dynamics. In addition, they can influence the vulnerability of freshwater bodies to climate change, due to their potential role in mitigating the effects of rising temperatures and extreme precipitation events.

The objective of this PhD. was to determine the role of riparian zones on the quality of freshwater, unraveling some factors of the interaction between climate change and changes in land use in drainage basins. We connect patterns observed in the field, with experimental analysis of soil processes, and experimental analyses of possible responses within waterbodies to potential indirect changes generated by riparian vegetation. For this, a review of national studies in direct or indirect relation to buffer zones was combined with intensive field monitoring, and experiments in mesocosms. The possible effects of climate change and land use on the retention of nutrients, mainly phosphorus, by riparian vegetation (comparing grassland, shrubland and native forest) on the Paso Severino Reservoir (Florida, Uruguay) were analyzed. In an annex experiment using farmland and native forest from the same fields, we simulated extreme warming and rainfall scenarios in the laboratory to assess nutrient release and changes in carbon fluxes. Likewise, we experimentally analyzed the responses of a primary aquatic producer (periphyton, in running waters) to factors conditioned by the presence of riparian vegetation: nutrient input and light availability, under flow pulses scenarios.

In the review we found diverse lines of research in connection with riparian vegetation; the theme in general is still incipient and underdeveloped at the national level, but growing rapidly. In the field sampling, we found large amounts of phosphorus (most of it in dissolved form) moving from crops to the Paso Severino Reservoir through surface and subsurface runoff water, and buffer zones playing a key role in its retention. According to our results, when phosphorus and nitrogen are considered simultaneously, a combination of herbaceous and woody vegetation would reach the maximum rate of nutrient retention. We found evidence that buffering capacity, and consequently the direct beneficial reduction of external load of nutrients, could be diminished under future climate scenarios, since the transportation of nutrients augments with the increase in the runoff rate caused by more intense rains. Furthermore, within the framework of this component, we develop innovative technologies for taking subsurface water samples.

Experiments showed warming could enhance the release of nutrients from agricultural soil. Regarding land use, in a soil waterlogging scenario, we found that phosphate release was

substantially higher in the cropland than in the forest. However, in precipitation events of greater magnitude in which the soil is effectively flooded, the benefits of the buffer zone were not so evident, since the release of phosphorus to the forest water reached values similar to those of the crop, and nitrogen was generally higher in the forest. This becomes particularly relevant if the load of nutrients arriving from the catchment basin to the buffer area is high and there is an accumulation of nutrients, since their cycling could be compromised. We recorded a shift from CO_2 sink to source in forest soil under conditions of warming and water availability, suggesting that global warming could accelerate the decomposition of soil organic carbon and potentially generate positive feedback on temperature increase.

In the artificial stream experiment, we show that the effects of flow pulses can have non-linear effects on periphyton in terms of biomass, as expected. However, the effects on the community composition varied strongly depending on the initial structure of the community (mediated by the base flow, the availability of light and nutrients). In a scenario of increased light, our results suggest greater periphyton growth and greater resistance against the natural control on its biomass exerted by flow pulses.

Throughout the thesis, evidence often highlighted the importance of maintaining or re-establishing buffer zones at the local level, to reduce the export of nutrients from agricultural soils. The role of buffer zones will be especially relevant in future scenarios of increased temperature and precipitation, although the effectiveness of their functions may also be compromised. This information is necessary to generate or adjust regulations, based on scientific knowledge, that enhance the resistance and resilience of aquatic ecosystems against different external disturbances such as the entry of nutrients or climate change. Specifically, the establishment of riparian vegetation in the water level fluctuation zone seems to be an effective method to reduce the input of nutrients to the studied Reservoir.

INTRODUCCIÓN

Efectos de las zonas buffer: desde la escala cuenca al cuerpo de agua

Las zonas buffer, o riparias, son las interfaces entre ecosistemas acuáticos y terrestres. Comprenden el área entre la cota baja y alta de fluctuación del nivel del agua, y la porción del paisaje terrestre donde la vegetación está influenciada por niveles freáticos elevados o inundaciones (Naiman & Décamps, 1997). Son áreas tridimensionales cuyos límites se extienden lateralmente más allá de la zona de inundación y hacia arriba en la bóveda formada por la vegetación (Gregory *et al.,* 1991; Verry *et al.,* 2004). Las zonas riparias interactúan y son moldeadas tanto por procesos fluviales y la geología de la zona, como por las tierras adyacentes (Auble *et al.,* 1994; Naiman & Décamps, 1997). Por ser áreas de transición y recibir influencia de dos ecosistemas tan distintos, se las considera entre los sistemas ecológicos de mayor complejidad (Décamps & Naiman, 1990). A pesar de la relativamente pequeña área que ocupan en la cuenca, promueven varios de los procesos que más resiliencia aportan a los ecosistemas acuáticos (sensu Holling, 1973). Han sido reconocidas por su valor en la regulación de procesos ambientales clave entre la cuenca y los sistemas de agua dulce, como el ciclado y la retención de nutrientes (Dosskey *et al.,* 2010), el aporte de materia orgánica (Vannote *et al.,* 1980), control de inundaciones (Croke *et al.,* 2017) y la regulación de la temperatura del agua e ingreso de luz (Broadmeadow *et al.,* 2011). Asimismo, una zona riparia en buen estado de conservación reduce la energía de erosión que ejerce el agua sobre las laderas (Naiman & Décamps, 1997), funciona como corredor ecológico para el continuo flujo de fauna (Schuler *et al.,* 2007) e incrementa notoriamente el atractivo del paisaje, aumentando en gran medida su valor estético y cultural (Wiens & Moss, 2005). Por la amplia gama de contribuciones positivas desde un punto de vista ambiental, una de las estrategias de restauración más frecuente en cuencas hidrográficas con calidad del agua degradada es la conservación y/o recuperación de las zonas riparias (Riis *et al.,* 2020; Urbanič *et al.,* 2022). Maximizar su funcionamiento es importante para mitigar los impactos ambientales generados como consecuencia de la intensificación agrícola y la contaminación industrial, los cambios en el uso del suelo, la modificación de los cursos de agua, y el cambio climático, sobre los ecosistemas acuáticos. En particular, la conservación o restauración de la vegetación riparia se utiliza globalmente como una buena práctica de gestión para la reducción de la carga externa de nutrientes que alcanzan las aguas superficiales.

La retención y remoción de nutrientes se da a través de mecanismos que involucran una alta deposición de partículas, absorción por parte de la vegetación y procesos microbianos del suelo (Dosskey *et al.,* 2010; Stutter *et al.,* 2009; Roberts *et al.,* 2013). Varios factores influyen sobre la eficiencia de las zonas riparias en la remoción de nutrientes. Entre los principales se encuentran el ancho de la región vegetada, el tipo de vegetación que la compone y la pendiente de las márgenes (Zhang *et al.,* 2010), aunque las diferentes efectividades reportadas en la bibliografía hacen que sea difícil generalizar sobre sus importancias relativas (Prosser *et al.,* 2020). Varios estudios reportan que la vegetación arbórea de raíces profundas es eficaz para atrapar nutrientes solubles, debido a su alta capacidad de infiltración, mientras que la vegetación herbácea es eficaz para atrapar sedimentos gruesos y nutrientes ligados a sedimentos (*Lee et al.,* 2000; Cao *et al.,* 2019). Por otra parte, se ha demostrado que áreas con vegetación arbórea pueden ser más efectivas en la remoción de nitrógeno y fósforo que aquellas con arbustos y pastizales (Lowrance *et al.,* 1984; Aguiar *et al.,* 2015; Walton *et al.,* 2020). En presencia de árboles, el suelo está protegido del impacto de las gotas de lluvia por interceptación del dosel y la capa de hojarasca, la tasa de infiltración es mayor y la erosión es menor (Aguiar *et al.,* 2015), con el efecto buffer fuertemente influenciado por variables propias del bosque (como la edad de los individuos e historia de disturbios) (de Souza *et al.,* 2013).

Las vías principales a través de las cuales los nutrientes son movilizados desde la cuenca y alcanzan los cuerpos de agua son la escorrentía superficial, el flujo sub-superficial y el flujo vertical hacia la zona de interacción con el agua subterránea. Cuál vía prevalecerá depende del patrón y duración de las precipitaciones, y del intervalo entre los eventos de precipitación (Haygarth *et al.,* 2000; Reid *et al.,* 2018). Por su parte, la composición del agua de escorrentía superficial y subsuperficial está estrechamente condicionada por el uso del suelo, la cubierta vegetal y las precipitaciones, que alteran las concentraciones, la reserva y las relaciones elementales entre los nutrientes (Groppo *et al.,* 2015; Hu & Li, 2019). Típicamente, el flujo superficial a través de la zona riparia facilita la retención de nutrientes unidos a partículas, mientras que el flujo subsuperficial a través de la zona de raíces facilita la absorción de nutrientes disueltos por parte de las plantas y la ocurrencia de transformaciones químicas (Dosskey, 2001; Hoffmann *et al.,* 2009). Estos movimientos conectan fuertemente las externalidades (i.e., consecuencias tanto positivas como negativas de los sistemas de producción que no son consideradas por los mercados, Pretty *et al.,* 2001) de las actividades llevadas a cabo en el medio terrestre con el ambiente acuático.

En estrecha relación con la reducción de nutrientes, la vegetación riparia, al aumentar la rugosidad de la superficie del suelo y la infiltración, disminuye los volúmenes y la velocidad del flujo superficial (Borin *et al.,* 2005). A través de esos efectos, diferencias relativamente pequeñas en la proporción del área riparia pueden regular significativamente los efectos de la agricultura sobre la escorrentía (Chase *et al.,* 2016). En los bosques en particular, una mayor capacidad de infiltración, recarga de aguas subterráneas por percolación vertical, y menor flujo superficial, llevan a un mayor flujo moviéndose sub-superficialmente y mayor almacenamiento de agua subterránea (Alvarenga *et al.,* 2017). Esa reducción en la escorrentía superficial puede minimizar las respuestas repentinas de los sistemas acuáticos frente a lluvias extremas, disminuyendo la frecuencia e intensidad de los pulsos de flujo (Chase *et al.,* 2016).

Por sus múltiples funciones, el ensamble de especies y los atributos de las comunidades acuáticas dependen, tanto de las características hidro-limnológicas de los cursos de agua, como de la cobertura vegetal de la zona litoral (Merritt *et al.,* 2010; **Ferreira *et al.,* 2019**; Dala-Corte *et al.,* 2020; **Barrios-Gómez *et al.,* 2022**), entre otros factores, que va a condicionar en parte la energía y materia que ingresa al cuerpo de agua desde el ambiente terrestre. La relevancia de la vegetación riparia radica en sus múltiples efectos sobre el control de la eutrofización, el régimen hidrológico y la cantidad de luz llegando a los ambientes acuáticos. Estos factores pueden condicionar en gran parte el crecimiento y configuración de los productores primarios (**Pacheco *et al.,* 2021**). Los grupos de productores primarios principales en sistemas de agua dulce son las macrófitas, el perifiton y el fitoplancton, con aportes diferenciales según se esté considerando sistemas lénticos o lóticos, la región litoral o central del cuerpo de agua (Wetzel, 1964) y la transparencia y profundidad del sistema (Liboriussen & Jeppesen, 2003).

Los organismos acuáticos están expuestos a rápidas fluctuaciones de los macro y micronutrientes presentes en la columna de agua, en gran parte por los aportes del agua de escorrentía durante eventos de precipitación (Mirás-Avalos *et al.,* 2014; Rier *et al.,* 2016). Asimismo, en los sistemas lóticos, el ingreso de escorrentía puede influir fuertemente sobre la velocidad de flujo, posiblemente alterando la configuración de las comunidades productivas (Bækkelie *et al.,* 2017; Wu *et al.,* 2019; Baattrup-Pedersen *et al.,* 2020). Un estudio de Rodríguez y colaboradores (2015) realizado en mesocosmos experimentales encontró que una mayor entrada de agua de escorrentía y un aumento de la temperatura tienen efectos fuertes y contrapuestos en la producción neta del hábitat pelágico y del hábitat litoral, resaltando la importancia de integrar información para realizar predicciones precisas a nivel de todo el ecosistema.

Así como los nutrientes y el flujo, el nivel de sombreado está estrechamente relacionado con la producción primaria acuática y el estado trófico (Burrell *et al.,* 2014). La fluctuación de la temperatura del agua y la radiación fotosintéticamente activa que ingresa, están condicionados en gran parte por la vegetación de las márgenes y sobre el cauce de los sistemas (Broadmeadow *et al.,* 2011; Julian *et al.,* 2011; Kristensen *et al.,* 2013), principalmente en ríos y arroyos. La magnitud y duración de la sombra dependerán de la altura de la vegetación, el nivel de cobertura, el ancho del canal y la época del año (Greenberg *et al.,* 2012; Li *et al.,* 2012), y en menor alcance del ancho de la zona riparia (DeWalle, 2010). En zonas subtropicales se ha encontrado una diferencia de hasta 10°C entre las temperaturas máximas de arroyos con y sin vegetación que bloquee la radiación solar (Marsh *et al.,* 2005). De esta forma, la vegetación riparia (principalmente arbórea) puede limitar el incremento en biomasa de las macrófitas y perifiton (Bunn *et al.,* 1998; Köhler *et al.,* 2010), y es efectiva en la mitigación del crecimiento de fitoplancton y generación de floraciones (Hutchins *et al.,* 2010). Asimismo, puede mitigar los aumentos en la temperatura del agua, evitando que superen valores umbrales de tolerancia de ciertos organismos acuáticos (Davies, 2010; Stewart *et al.,* 2013). Cuando la vegetación riparia es removida se perturban las condiciones naturales hidrológicas y de acceso a nutrientes y luz. Estos factores actuando en sinergia pueden causar un incremento de biomasa de productores primarios, así como cambios en su composición específica y funcional, las cuales directa o indirectamente provocan cambios en los demás componentes del ecosistema.

Zonas buffer en un contexto de cambios globales

A nivel global, las crecientes actividades económicas que se desarrollan en las riberas de los cursos de agua han traído como resultado un fuerte impacto sobre la vegetación riparia y el funcionamiento general de estos ecosistemas (Singh *et al.,* 2021). Como consecuencia, surgen innumerables amenazas y modificaciones en la dinámica de los cursos de agua, ya que incluso impactos localizados en una región específica pueden tener efectos diferidos en el tiempo o el espacio (Allan, 2004). La agricultura aparece como el principal impulsor del cambio de uso de la tierra, y su intensificación en las últimas décadas está aumentando aún más la transferencia difusa de nutrientes a los cuerpos de agua superficiales (Strayer *et al.,* 2003; Allan, 2004; Foley *et al.,* 2005; Bender *et al.,* 2018). Sumado a la creciente exportación de nutrientes provenientes de la cuenca, para maximizar el área de terreno destinada a la producción, frecuentemente se remueve, de manera parcial o total, la vegetación riparia y se convierte en área de cultivos (Feijoó *et al.,* 2012; Celentano *et al.,* 2016; Lilli *et al.,* 2020), intensificando los efectos sobre los ecosistemas acuáticos.

Uno de los problemas más extendidos de los ecosistemas de agua dulce en todo el mundo es la eutrofización cultural: la contaminación antrópica por nutrientes, generalmente fósforo, pero también nitrógeno (Smith *et al.,* 1999; Withers *et al.,* 2014; Mekonnen & Hoekstra, 2015; 2018; **Fig. 1**). Este fenómeno degrada la calidad del agua, disminuye la biodiversidad y altera la estructura y composición de las comunidades biológicas (evidencias nacionales: Conde *et al.,* 2002; Mazzeo *et al.,* 2003; Pacheco *et al.,* 2012; Chalar *et al.,* 2013; Benejam *et al.,* 2016), limitando además los posibles usos antrópicos que se le pueden dar a estos ecosistemas.

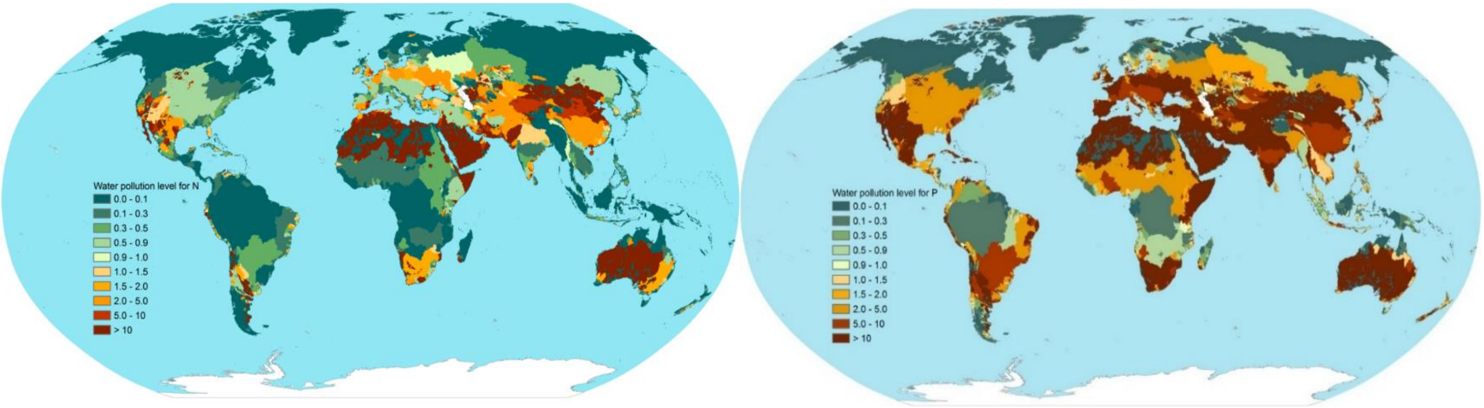


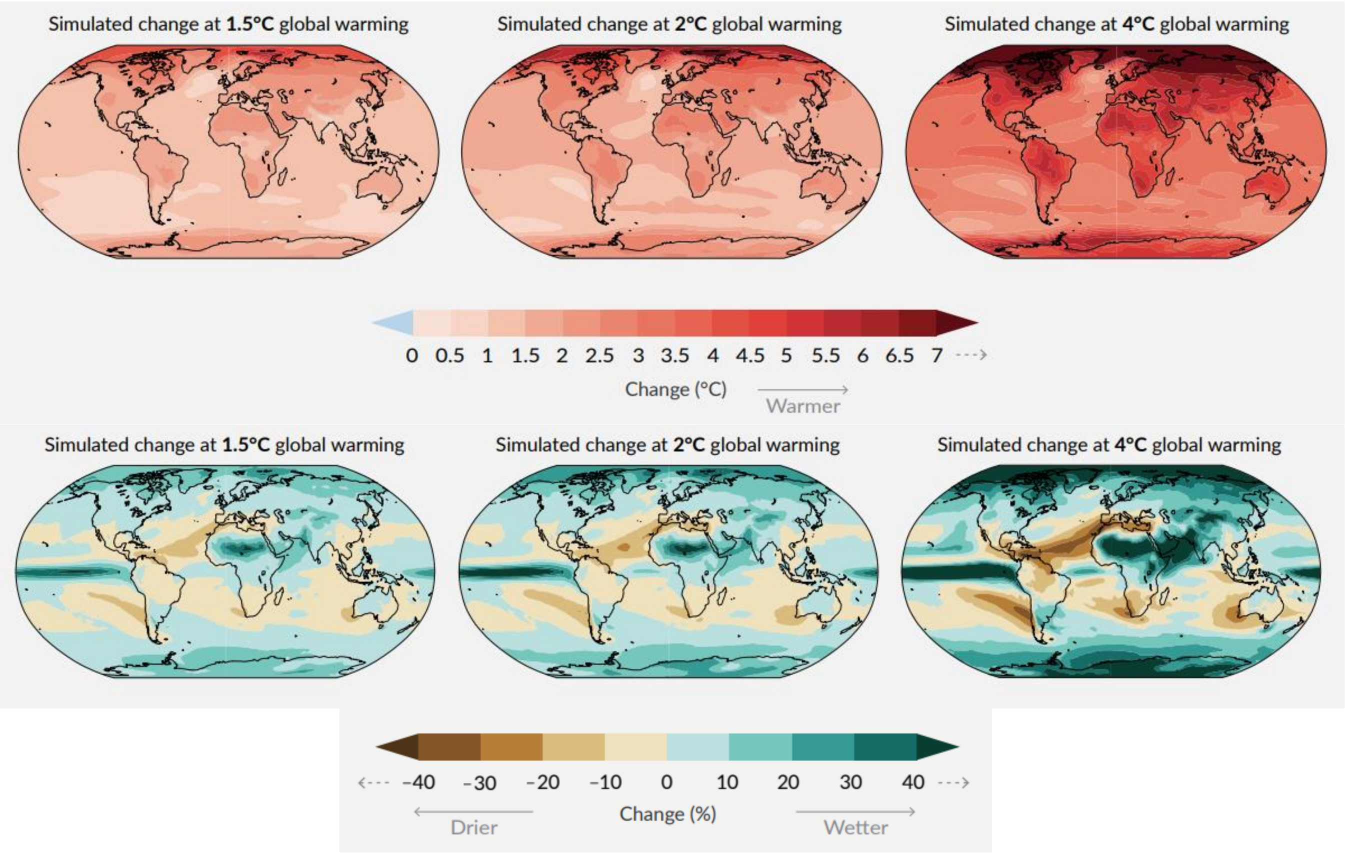
Figura 1. Mapa global de nivel de contaminación (por su sigla en inglés: Water pollution level) por fósforo (P) y nitrógeno (N). Los autores estimaron las cargas antropogénicas de P y N en el agua dulce, y relacionaron por cuenca fluvial con la escorrentía para calcular el nivel de contaminación del agua por cuenca. El WPL mide la capacidad de asimilación de residuos de las cuencas hidrográficas. Si es mayor que 1, entonces los contaminantes emitidos exceden la capacidad máxima de asimilación de la cuenca bajo un estándar de calidad de agua definido y violan la seguridad ambiental del agua dulce. Figuras extraídas de: Mekonnen & Hoekstra, 2015; 2018.

Monitoreos a largo plazo han demostrado que la actividad agrícola en general modifica los procesos de escorrentía y erosión del suelo, acentuando la pérdida de fósforo en comparación con ambientes boscosos (Verheyen *et al.,* 2015). En parte, esto se da porque las superficies más lisas, como son las áreas cultivadas, promueven la escorrentía (Simmonds *et al.,* 2016, Zhao *et al.,* 2018). Por su parte, la estratificación vertical de los nutrientes añadidos por fertilización y no incorporados por los cultivos (con mayores concentraciones principalmente en los primeros 2.5 cm del suelo), hace que las primeras capas sean las más influyentes en la composición del agua de escorrentía (Sharpley *et al.,* 2000; Tiessen *et al.,* 2010; Rowe *et al.,* 2016; Baker *et al.,* 2017).

A la intensificación en el uso del suelo y sus múltiples consecuencias se suma un escenario actual de cambios climáticos, bajo el cual se espera que varios procesos físicos, químicos y biológicos cambien en dirección y magnitud, afectando los vínculos entre el ambiente terrestre y acuático. Investigación científica internacional y nacional sostiene que el aumento de la temperatura ambiente y los cambios en el régimen de precipitaciones (IPCC, 2021; **Fig. 2**) en general actúan de manera sinérgica con el aumento de nutrientes (Moss *et al.,* 2011; Meerhoff *et al.,* 2012; Meerhoff *et al.,* 2022). Entre otros factores, esto es debido a alteraciones en la biodisponibilidad y al aumento del transporte de nutrientes (especialmente fósforo) entre los entornos terrestre y acuático (Fey *et al.,* 2015; Gong *et al.,* 2015; Steinauer *et al.,* 2015). La vegetación riparia, al incidir sobre los efectos directos e indirectos del clima sobre la eutrofización, y los regímenes térmicos e hidrológicos, puede conducir a cambios a nivel poblacional y comunitario acuáticos (ej. Samways *et al.,* 2011; Cross *et al.,* 2013; **Figueiredo *et al.,* 2019**; Montag *et al.,* 2019). La presencia de vegetación riparia (principalmente arbórea) puede alterar los regímenes térmicos acuáticos al disminuir las temperaturas máximas, la variabilidad, y la frecuencia y duración de las temperaturas elevadas, principalmente en las estaciones cálidas (Roon *et al.,* 2021). Asimismo, reduce la radiación fotosintéticamente activa en el agua, que junto con el calentamiento del agua puede causar un incremento de la biomasa de los productores primarios, así como cambios en su composición específica y funcional, los cuales directa o indirectamente repercuten en los demás componentes del ecosistema (Julian *et al.,* 2011). En ciertas condiciones, la sombra puede desempeñar un papel más importante en la amortiguación de los efectos de la eutrofización en arroyos que el manejo de los nutrientes (Schiller *et al.,* 2007). Por su reconocido rol, se ha propuesto aumentar el sombreado como medida de adaptación a cambios en el clima; sin embargo, deben tenerse en cuenta los efectos colaterales que puede tener sobre las comunidades acuáticas y el funcionamiento ecosistémico (Thomas *et al.,* 2016).

Además del calentamiento, se prevé que los eventos de precipitación extrema aumenten su frecuencia a nivel regional en el contexto de cambio climático (Haylock *et al.,* 2006; Westra *et al.,* 2014; IPCC, 2021; **Fig. 2**). Un mayor volumen y/o una mayor intensidad de eventos de lluvia probablemente aumentarán el flujo de nutrientes a través de la cuenca, intensificando su transferencia al ambiente acuático (Gao *et al.,* 2014; Ockenden *et al.,* 2017). En particular, se espera una mayor carga de fósforo y nitrógeno en los cuerpos de agua, transportados por escorrentía superficial y subsuperficial, con variabilidad estacional dependiendo del clima local (Jeppesen *et al.,* 2009; Jeppesen *et al.,* 2011; Ockenden *et al.,* 2016). Estas mayores cargas podrían promover aún más las floraciones algales y de cianobacterias (Paerl & Huisman, 2008; González-Piana *et al.,* 2017; Haakonsson *et al.,* 2017; Meerhoff *et al.,* 2022). Varios experimentos han demostrado que la dinámica del fósforo en suelo cultivado, y la comunidad microbiana asociada, dependen en gran medida del régimen de agua al que esté sometido el suelo (i.e. ciclos de secado y re-humedecimiento pueden aumentar significativamente las fracciones de fósforo lábil en mayor medida que el humedecimiento continuo) y del tipo de fertilizante que se aplique (mineral u orgánico) (Forber *et al.,* 2017; Sun *et al.,* 2017). Por lo anterior, la concentración de elementos en el suelo y en el agua de escorrentía no solo depende del uso del suelo, sino que está estrechamente vinculada al clima (principalmente a los eventos de lluvia) (Pote *et al.,* 1996). Se demostró en un estudio con diversos fertilizantes comercializados comúnmente que, del fósforo aplicado superficialmente, aproximadamente un 20% se pierde por escorrentía en el primer evento de precipitación (Smith *et al.,* 2016). Al comienzo de un evento, se da un transporte inicial de fósforo disuelto rápido y de corta duración, producto de la escorrentía superficial desde el ambiente terrestre, mientras que un transporte retrasado indicaría un movimiento de flujo subterráneo (Feijoó *et al.,* 2018).

Existe un conocimiento cada vez mayor sobre la liberación de fósforo en su forma disuelta desde los suelos agrícolas (ej. Baker *et al.,* 2017; Reid *et al.,* 2018), pero escasea sobre la liberación frente a distintas condiciones climáticas (Maranguit *et al.,* 2017). En experimentos a corto plazo inundando suelo de uso agrícola se encontró que la tasa potencial de movilización de fósforo (i.e., la transferencia desde el sistema suelo-planta al agua de escorrentía superficial) aumenta al aumentar la temperatura. Por ende, el riesgo de eutrofización por dicho proceso probablemente sería mayor en las estaciones cálidas (Tang *et al.,* 2016; Yu *et al.,* 2016) o a futuro por el calentamiento climático, así como sucede en la interfase sedimento-agua (Jeppesen *et al.*, 2009; Wang *et al.*, 2013).



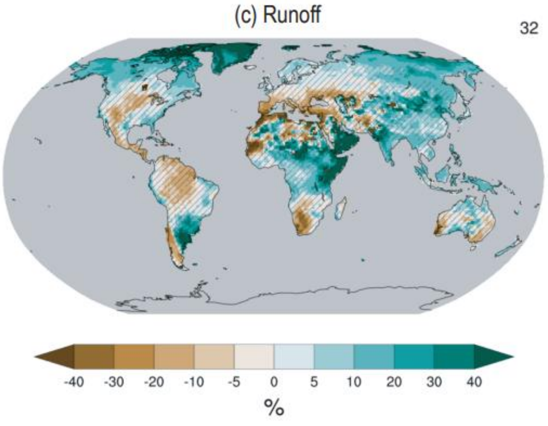


Figura 2. Cambios simulados de la temperatura media anual (°C) (arriba), y cambio en la precipitación (%) (medio), a niveles de calentamiento global de 1.5 °C, 2 °C y 4 °C (cambio promedio de 20 años de la temperatura superficial global en relación con 1850-1900). Debajo: Cambios medios anuales proyectados (%) a largo plazo (2081-2100) en relación con el presente (1995-2014) en el escenario de emisiones SSP2-4.5 para escorrentía total. Figuras extraídas de: IPCC, 2021.

Debido a su posición topográfica en el paisaje, las zonas riparias están generalmente muy expuestas a eventos climáticos extremos, como inundaciones y sequías (Capon *et al.*, 2013), que varían enormemente las condiciones de temperatura y disponibilidad hídrica. Sin embargo, es particularmente escasa la investigación sobre la liberación de fósforo desde suelo con coberturas no agrícolas y su relación con el cambio climático, aun sabiendo que las dinámicas de los procesos de escorrentía y erosión del suelo se relacionan fuertemente con la variabilidad climática (Duncan *et al.,* 2015). El calentamiento puede alterar el ciclo del fósforo y nitrógeno a través de múltiples factores, por ejemplo, al influir en el balance hídrico de los suelos, los subsidios terrestres, la actividad de la comunidad microbiana y los procesos de inmovilización y movilización química, aunque muchos de los mecanismos siguen sin estar claros (ej. Fey *et al.,* 2015; Condon *et al.,* 2020). La importancia del tipo de cobertura vegetal en el terreno radica en su estrecha relación con dichos factores. En particular, frente a un aumento de temperatura, una mayor riqueza de especies de plantas cubriendo el suelo puede incidir sobre el contenido de agua, potencialmente mitigando su pérdida (Steinauer *et al.,* 2015).

En parcelas con vegetación herbácea sometidas a distintos regímenes de temperatura se encontró que en escenarios más cálidos se reduce el fósforo disponible en el suelo, posiblemente por un estímulo de la producción primaria y una mayor actividad microbiana (Gong *et al.,* 2015). Sin embargo, según evidencia reciente, existe riesgo de que a largo plazo el fósforo sea liberado en su forma disuelta desde áreas de vegetación riparia cuando el suelo se vuelva a humedecer, principalmente luego de períodos secos o durante períodos prolongados de saturación de agua (Gu *et al.,* 2017), que podrían darse como consecuencia de un incremento en los eventos de precipitación extremos. Asimismo, en experimentos de microcosmos usando suelo extraído de áreas riparias, se encontró que la pérdida de fósforo al rehumedecer el suelo puede ser promovida al aumentar la temperatura, efecto probablemente mediado por procesos biológicos (Stutter *et al.,* 2009).

Esto es especialmente relevante en escenarios donde la carga de nutrientes llegando desde la cuenca de captación es alta, y las tasas de inmovilización y transformación de fósforo y nitrógeno a través de procesos biológicos (es decir, mineralización microbiana, desnitrificación y asimilación por vegetación) son insuficientes para retenerlos (Stutter *et al.,* 2012; Valkama *et al.,* 2019). El exceso de nutrientes puede conducir a un enriquecimiento de los suelos riparios con formas lábiles, en algunos casos en mayor medida que en los campos agrícolas adyacentes. Esto sugiere que los suelos en las zonas buffer saturadas pueden potencialmente convertirse en una fuente (y no sumidero) importante de nutrientes inorgánicos y orgánicos (Dodd & Sharpley, 2016; Young & Ross, 2018; Concepción *et al.,* 2021; Talbot *et al.,* 2021), causando riesgos especialmente altos para los ambientes acuáticos durante eventos hidrológicos episódicos como flujo alto, deshielo, y erosión de márgenes (Dosskey *et al.,* 2010; Vidon *et al.,* 2010). Estos eventos, que probablemente aumenten su frecuencia con el cambio en el clima, pueden aumentar la disponibilidad de agua en los suelos causando su saturación o incluso inundación, por períodos muy variables en duración. Una de las principales consecuencias del anegamiento o inundación del suelo es la alteración de sus características físicas, químicas y biológicas, y la menor capacidad para proporcionar un medio óptimo para el crecimiento de las plantas terrestres (revisión reciente en Manik *et al.,* 2019). Esto es especialmente relevante en las zonas de ribera aledañas a embalses, que tienen una dinámica hidrológica particular y un régimen hídrico frecuentemente regulado de manera artificial que, junto con las actividades antrópicas desarrolladas en la cuenca, determinan fuertemente las propiedades del suelo (Ye *et al.,* 2019; Wang *et al.,* 2021).

Posibles feedbacks mediados por las zonas buffer

Las emisiones de gases de efecto invernadero (GEI) de los suelos cambian en respuesta a los cambios climáticos. Las inundaciones pueden aumentar las emisiones netas de GEI del suelo (CH_4 y N_2O), debido a los cambios en el potencial redox del suelo y en la actividad microbiana (Hou *et al.,* 2000). A su vez, el calentamiento del suelo acelera la descomposición de la materia orgánica, lo que aumenta la liberación de CO_2 a la atmósfera (Davidson & Janssens, 2006). En los suelos riparios, el almacenamiento y la disponibilidad elevados de nutrientes influyen en la productividad biológica y, en consecuencia, pueden afectar los flujos de CO_2 (Petrone *et al.,* 2008; Chen *et al.,* 2022), junto con los cambios estacionales en las condiciones biogeoquímicas (Vidon *et al.,* 2014). Los impactos combinados de las inundaciones y el calentamiento pueden provocar una gran pérdida de C gaseoso (CO_2 y CH_4) desde suelos buffer enriquecidos en materia orgánica, en parte debido a la senescencia de las plantas y a las grandes cantidades de material en descomposición (Sánchez-Rodríguez *et al.,* 2019). En las áreas de fluctuación del nivel del agua, los flujos de CO_2 pueden variar en función de los tipos de cobertura terrestre adyacentes, con más emisiones de CO_2 cerca de bosques que cerca de pastizales, probablemente porque los bosques tienen más materia orgánica (Almeida *et al.,* 2019). De esta forma se da un posible feedback positivo entre el cambio climático y los flujos de carbono del suelo, impulsado por el aumento de la descomposición microbiana y la consecuente pérdida de carbono bajo escenarios de cambios en el clima y en el uso de la tierra. Sin embargo, aún no hay consenso sobre cómo la temperatura y el enriquecimiento por nutrientes del suelo afectan las respuestas durante eventos prolongados de saturación de agua e inundaciones de corto plazo.

Problema de investigación

La influencia de las zonas riparias sobre la dinámica de los ecosistemas acuáticos continentales, y en particular sobre la calidad del agua, está ampliamente documentada y es un tema que en los últimos años ha cobrado relevancia nacional dentro del ámbito científico y de gestión ambiental. Sin embargo, aún es escasa la información generada en climas subtropicales, como los de nuestro país, donde la estructura comunitaria y las dinámicas biológicas y de nutrientes de los ecosistemas lénticos y lóticos son diferentes a los tropicales y templados (ej. Meerhoff *et al.,* 2007, 2012; Teixeira de Mello *et al.,* 2009, 2012; Goyenola *et al.,* 2015, 2020). Esta información es necesaria para generar o ajustar regulaciones basadas en conocimiento científico, que restrinjan el uso y la extracción de la vegetación riparia, y potencien la resistencia y resiliencia de los ecosistemas (Holling 1973) frente a diferentes perturbaciones externas. Incluso en lugares ya degradados, la calidad del agua y del hábitat pueden mejorarse al restaurar la vegetación de la zona riparia y retornarla a su dinámica natural (Kozlowski *et al.,* 2016).

A nivel nacional, la intensificación del sistema productivo que ha tenido lugar principalmente en zonas agrícolas en los últimos 30 años, se ha visto asociada a un aumento en el aporte de nutrientes, y en particular de fósforo, a los cuerpos de agua (Arocena *et al.,* 2008; Goyenola *et al.,* 2015; Chalar *et al.,* 2017; Díaz *et al.,* 2021; Lucas *et al.,* 2022). Arocena y colaboradores (2018) encontraron, basándose en el análisis conjunto de 28 sub-cuencas de la cuenca del Río Santa Lucía, que la intensidad de la agricultura es el factor más importante asociado con la calidad del hábitat y del agua en arroyos. Los desechos animales y los fertilizantes agrícolas son, en muchas cuencas clave, los principales responsables de las altas cargas de nutrientes en los ecosistemas acuáticos (DINAMA, 2011), y los parámetros físico-químicos del agua pueden variar acompañando períodos puntuales del proceso de producción agropecuaria (Tommasina, 2012). Los planes de uso y manejo de suelos afectan enormemente la dinámica de los nutrientes. Un sistema de cultivo que incluya rotación con pasturas, por ejemplo, puede aumentar en un 40% la infiltración de agua en el suelo y así reducir la pérdida de nutrientes hacia el ambiente acuático (Lizarralde *et al.,* 2016). Barreto *et al.* (2022) al comparar distintas prácticas de manejo del suelo, encontraron los valores más bajos de escorrentía en pradera natural (donde el suelo mantiene sus propiedades físicas y favorece una mayor infiltración de agua), mientras que en las rotaciones cultivos-pastura, el pisoteo animal compacta el suelo y hay mayor escorrentía.

En los ecosistemas acuáticos superficiales de Uruguay, el fósforo en el agua se encuentra generalmente en su forma biodisponible (Barreto *et al.,* 2017), promoviendo el crecimiento de plantas acuáticas y más frecuentemente favoreciendo floraciones de microalgas y cianobacterias (Aubriot *et al.,* 2005; Piccini *et al.,* 2011; Aubriot & Bonilla, 2012; Bonilla *et al.,* 2015; Kruk *et al.,* 2015; Crisci *et al.,* 2017; González-Madina *et al.,* 2017; Kruk *et al.,* 2017; Segura *et al.,* 2017; Alcántara *et al.,* 2018; Bonilla *et al.,* 2021) y constituyendo un riesgo potencial para la salud humana y animal (Vidal *et al.,* 2017; Pacheco *et al.,* 2021). El fosfato puede representar en áreas de intensa actividad agrícola de la cuenca del Río Santa Lucía más del 85% del fósforo total (Goyenola *et al.,* 2015). Las altas concentraciones de fósforo en esta forma en el agua podrían evidenciar el uso de fertilizantes en cantidades mayores a las que el suelo puede retener (Aubriot *et al.,* 2017), o un desacople de la práctica agrícola respecto de las condiciones meteorológicas. Aunque recientemente se ha cuestionado el rol de los nutrientes de origen agrícola como causa principal de las floraciones de cianobacterias, la copiosa evidencia nacional e internacional lo hace incuestionable (Beretta-Blanco & Carrasco-Letelier, 2021; **Alcántara *et al.,* 2021**).

Gran parte de esta tesis se centró directa o indirectamente en el Embalse de Paso Severino (Florida) sobre el Río Santa Lucía. Este embalse es la principal reserva de agua dulce del Uruguay, y proporciona agua potable a casi el 60% de la población del país. La intensificación de las actividades productivas ha comprometido seriamente la calidad del agua de este río (Somma *et al.,* 2021), estimándose que el 80% de la contaminación por nutrientes se debe a fuentes difusas provenientes de actividades agrícolas (Manta *et al.,* 2013). Un dramático evento de mal olor y sabor causado por cianobacterias (no tóxicas) comprometió el acceso de agua a Montevideo y el área metropolitana (2013). A partir del mismo, se tomaron varias medidas de protección y rehabilitación en la cuenca, a través del "Plan de acción para la protección de calidad ambiental y fuentes disponibles de agua potable en la cuenca de Santa Lucía", el mayor plan de recuperación de la calidad del agua a nivel de cuenca implementado hasta el momento (DINAMA, 2013). La protección y recuperación de las zonas riparias fue una de las varias medidas adoptadas en esta cuenca. La ganadería fue excluida mediante el alambrado de la zona del predio inundable perteneciente a

OSE (organismo estatal responsable del abastecimiento de agua potable), permitiendo el crecimiento y regeneración de la vegetación. Como resultado, las márgenes del reservorio actualmente tienen tres tipos de cobertura que prevalecen: pastizal natural (sin vegetación arbórea), pastizal arbustizado (de ahora en más arbustal) y bosque nativo (ya establecido), con las que se trabajó en distintos componentes del proyecto. Estas coberturas son representativas del tipo de vegetación terrestre en Uruguay, donde se destaca en área el pastizal. Las especies vegetales de gran porte están restringidas principalmente a zonas de quebradas, serranías y las riberas de sistemas dulceacuícolas, siendo este último tipo de bosque uno de los de mayor cobertura y ocurrencia en todo el país (Panario *et al.,* 2011; Brazeiro, 2014; Altesor *et al.,* 2019; Bernardi *et al.,* 2022).

Las prácticas de conservación y manejo, a fin de mitigar las altas cargas de contaminantes, deben tener como objetivo minimizar el transporte de nutrientes y sedimentos, y maximizar la efectividad de las zonas de amortiguamiento riparias (Yasarer *et al.,* 2017; Wagena & Easton, 2018; Martínez-Mena *et al.,* 2020). Este es un objetivo extremadamente difícil ya que existe una gran variabilidad de observaciones sobre la dinámica y el transporte de nitrógeno y fósforo entre el ambiente terrestre y acuático, y es aún más complejo si consideramos el escenario actual de cambio climático y en el uso de la tierra. Por ende, es necesario combinar estudios de monitoreo en campo con instancias experimentales a fin de dilucidar el gran nivel de complejidad y la diversidad de escalas actuando de forma simultánea.

OBJETIVOS, HIPÓTESIS Y APROXIMACIONES

Objetivo general

El objetivo de este doctorado fue determinar el rol de las zonas riparias sobre el funcionamiento de ecosistemas acuáticos, particularmente sobre la calidad del agua dulce superficial, analizando algunos mecanismos directos e indirectos, así como posibles efectos de la interacción entre el cambio climático y cambios en el uso de la tierra en las cuencas de drenaje.

Se buscó conectar patrones en campo a escala de cuenca, con análisis de procesos a nivel de laboratorio, y análisis experimentales de las posibles respuestas a nivel acuático frente a cambios indirectos potencialmente generados por la presencia y ausencia de vegetación riparia. Asimismo, se buscó contribuir al diseño o ajuste de medidas de restauración apropiadas a los sistemas acuáticos eutróficos del Uruguay.

Objetivos, hipótesis, y aproximaciones específicas

O1. Identificar y analizar las distintas problemáticas de calidad de agua superficial del Uruguay, haciendo énfasis en los estudios que analicen, directa o indirectamente, el rol buffer de la vegetación riparia. Este objetivo se desarrolló a través de la revisión de trabajos nacionales.

H1. La aplicación de agroquímicos y el aumento del área destinada a la actividad agropecuaria, debido a la intensificación productiva llevada a cabo en distintas cuencas del país ejerce actualmente mayores presiones sobre los ecosistemas de agua dulce superficiales que otras actividades antrópicas, lo que se espera ver reflejado en la literatura actualizada. Por su rol como barreras de protección de los sistemas acuáticos, es de interés generar conocimiento local en cuencas con gran presión productiva donde podrían contribuir a la conservación o recuperación de cuerpos de agua deteriorados, a través de la mitigación del proceso de eutrofización y de sus sinergias con el cambio climático.

A1. Se realizó una búsqueda bibliográfica de publicaciones exclusivamente nacionales sobre problemáticas asociadas a sistemas acuáticos continentales. En esta tesis se presenta únicamente la información generada a nivel nacional en relación directa o indirecta a las zonas buffer (Capítulo 1).

O2. Analizar los efectos de distintos tipos de vegetación riparia en una misma cuenca (pastizal, arbustal y bosque nativo) sobre las características físico-químicas del agua de escorrentía superficial y subsuperficial desde cultivos adyacentes, y su relación con distintas condiciones climáticas (de temperatura y precipitaciones).

H2. La vegetación riparia promueve la retención y remoción de nutrientes provenientes de predios agrícolas a través de mecanismos que involucran una alta deposición de partículas, absorción por parte de la vegetación y procesos microbianos del suelo. Los distintos tipos de vegetación riparia y sus atributos particulares modifican las características físico-químicas del agua de escorrentía superficial y subsuperficial, ya que varían en la magnitud y forma de absorción y/o infiltración de sedimentos, nutrientes y micronutrientes provenientes del área de drenaje, al mismo tiempo que aportan materia orgánica. Se espera que el bosque retenga una mayor cantidad de nutrientes del agua de escorrentía superficial y subterránea que la cubierta de pastizales (y un efecto intermedio en el arbustal), por tener mayor tasa de infiltración, un sistema de raíces profundas, mayor contenido de materia orgánica en el suelo y una gran biomasa y diversidad microbiana. En relación a la variabilidad climática, se espera que una mayor intensidad de las lluvias reduzca el efecto "amortiguador" de la vegetación al aumentar el transporte de sustancias entre el medio terrestre y el acuático, con efectos de mayor magnitud en las zonas riparias herbáceas (debido en gran parte a la menor protección del impacto directo de precipitación).

A2. Este análisis se realizó en campo en la cuenca del Embalse de Paso Severino. Evaluamos *in situ* el agua superficial y subsuperficial de tres franjas buffer con vegetación diferente y sus respectivos cultivos adyacentes ubicados pendiente arriba en la cuenca (pradera mejorada durante el período de estudio). El monitoreo tuvo una duración de un año, a fin de probar la capacidad de retención de nutrientes bajo un amplio rango de precipitaciones naturales. Para la colecta de agua subsuperficial se utilizaron dispositivos de diseño propio (Capítulo 2 y 3).

O3. Determinar si, y cómo, la magnitud de liberación del fósforo y las emisiones de CO_2 desde suelos provenientes de zonas con dos tipos de cobertura (suelo agrícola y bosque nativo), es afectada por cambios en la temperatura ambiente (control y elevada) y el régimen de precipitación.

H3. La anegación e inundación temporarios del suelo luego de eventos de precipitación extremos alteran la actividad microbiana y los mecanismos actuando (ya que las tolerancias al exceso de agua y condiciones anaeróbicas varían entre la comunidad) y, por lo tanto, producen cambios en el ciclo de nutrientes, con más P liberado en el agua y proporciones modificadas en las formas del nitrógeno. Por su parte, un aumento de la temperatura acelera las reacciones químicas y biológicas del suelo y, por lo tanto, la descomposición de la materia orgánica, lo que intensificará la liberación de nutrientes frente a eventos de anegamiento o inundación de corto plazo. Para las emisiones de CO_2 se espera que aumenten en anegamiento, pero disminuyan en inundación (por reducción de la respiración en anaerobia), y que la emisión sea mayor en suelos bien drenados. Además, hipotetizamos que el uso del suelo influye fuertemente en la disponibilidad de nutrientes en las capas superficiales del suelo (en los cultivos debido a la fertilización y las prácticas de manejo, y en los bosques debido a la posible retención y enriquecimiento de nutrientes). Las concentraciones y dinámicas específicas de cada cobertura condicionan la liberación de nutrientes al suelo y al agua de inundación.

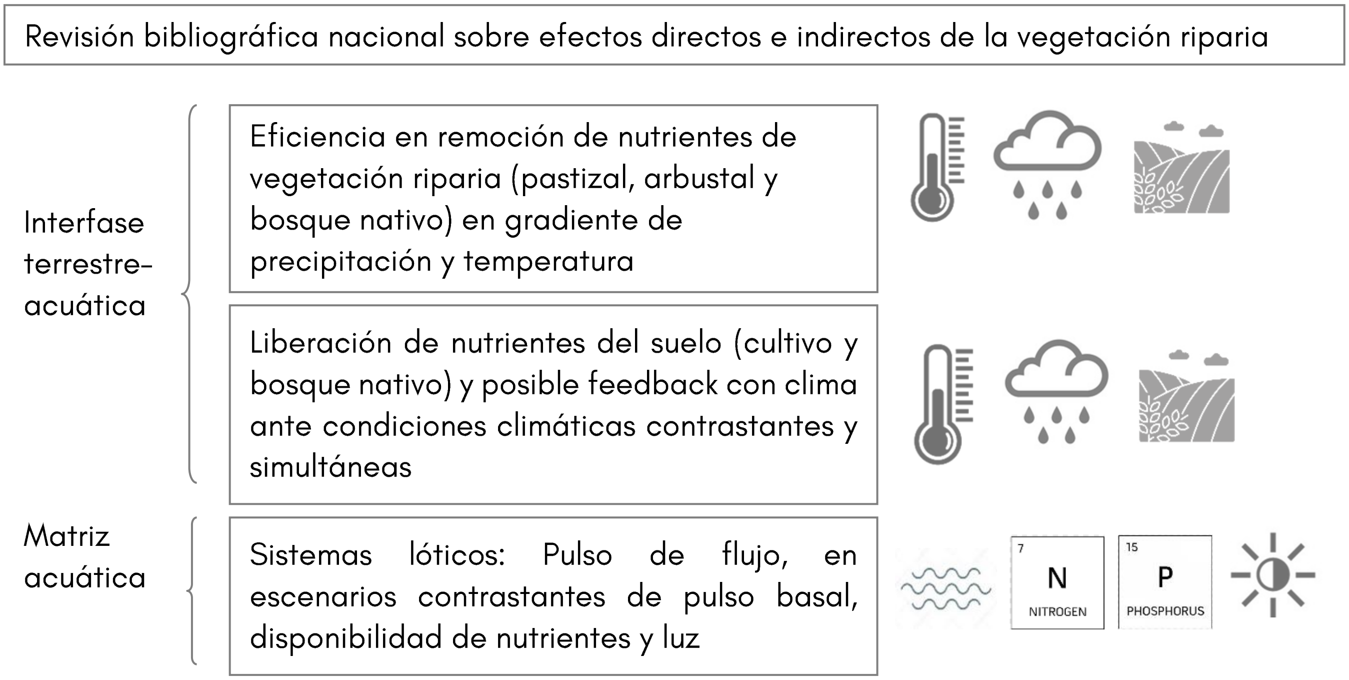
A3. Este análisis se desarrolló de manera experimental en laboratorio dentro de cámaras climáticas simulando condiciones de anegamiento e inundación de suelo agrícola y de bosque, en dos escenarios de temperatura ambiente (temperatura control y elevada en 3 grados) (Capítulo 4).

O4. Analizar la interacción entre procesos promovidos directa e indirectamente por las zonas riparias y la precipitación (a través de la ocurrencia de pulsos de flujo) sobre el desarrollo de la comunidad perifítica (productor primario típico de sistemas lóticos). Nos centramos en escenarios contrastantes de flujo basal, disponibilidad de nutrientes y luz, los tres factores también condicionados en gran parte por la presencia o ausencia de vegetación riparia.

H4. Pusimos a prueba dos hipótesis: (1) a flujos basales más bajos, un pulso de flujo promueve un mayor crecimiento del perifiton a través de una mayor transferencia de nutrientes, mientras que, a flujos basales más altos, un pulso de flujo reduce la biomasa del perifiton debido a una mayor remoción de organismos. Estos mecanismos contrastantes que actúan a flujo bajo y alto probablemente conducen a una respuesta unimodal de la biomasa perifítica a los pulsos de flujo, (2) la alta disponibilidad de nutrientes y luz (simulando ausencia de vegetación riparia) promueve un mayor crecimiento del perifiton e impulsa cambios en la composición a través del recambio de especies, probablemente compensando los efectos negativos potenciales de los mayores pulsos de flujo.

A4. Este análisis se realizó en mesocosmos experimentales de aguas corrientes ubicados al aire libre, donde se estudió el efecto de pulsos de flujo sobre la estructura de las comunidades desarrolladas bajo escenarios contrastantes de flujo basal de agua, enriquecimiento de nutrientes y disponibilidad de luz (Capítulo 5).

RESUMEN GRÁFICO



Revisión bibliográfica nacional sobre efectos directos e indirectos de la vegetación riparia

Interfase terrestre-acuática

- Eficiencia en remoción de nutrientes de vegetación riparia (pastizal, arbustal y bosque nativo) en gradiente de precipitación y temperatura
- Liberación de nutrientes del suelo (cultivo y bosque nativo) y posible feedback con clima ante condiciones climáticas contrastantes y simultáneas

Matriz acuática

- Sistemas lóticos: Pulso de flujo, en escenarios contrastantes de pulso basal, disponibilidad de nutrientes y luz

RESULTADOS PRINCIPALES

CAPÍTULO 1. REVISIÓN SOBRE PROBLEMÁTICAS ASOCIADAS A LA CALIDAD DE AGUA DULCE EN URUGUAY CON ÉNFASIS EN ZONAS BUFFERS

Los ecosistemas de aguas quietas y de aguas corrientes, a pesar de grandes diferencias en su funcionamiento, comparten gran parte de las problemáticas. Estas aguas son de gran importancia a nivel social y cultural como lugares de recreación y disfrute paisajístico, así como para el desarrollo de emprendimientos turísticos en todo el país. Como proveedores de recursos para la población se destaca el suministro de agua para potabilizar, agua para riego agrícola y uso en actividades productivas (consumo animal), y como fuente de diversos recursos pesqueros. A nivel ecosistémico, su integridad promueve el mantenimiento de altos índices de biodiversidad, amortiguación frente a inundaciones y eventos climáticos extremos, así como el ciclado de nutrientes y otras sustancias que ingresan desde la cuenca de drenaje, entre otros procesos clave.

En Uruguay existen muestreos sistemáticos de la calidad del agua de varios ecosistemas acuáticos superficiales (y en algunos casos de sus cuencas) por parte de diversos organismos estatales, pero aun se tiene un conocimiento fragmentado del estado actual de los recursos acuáticos. Las crecientes actividades antrópicas llevadas a cabo en distintas cuencas del país ejercen presiones sobre los ecosistemas de agua dulce, y el desafío en la actualidad consiste en lograr mantener su integridad ecológica y calidad del agua o promover su restauración, según sea el estado actual de deterioro y las predicciones de cambio a futuro (Meerhoff & Bernardi, 2018; Rodríguez-Gallego *et al.*, 2019).

A partir de una extensa revisión bibliográfica de publicaciones exclusivamente nacionales sobre sistemas acuáticos continentales construimos un diagrama de flujo detallando: factores o variables directas que afectan el estado de los componentes del medio ambiente, de manera individual o colectiva y de origen antrópico o por procesos naturales (causas secundarias y causas primarias), la situación en que se encuentran los componentes del medio ambiente (estado actual según la información publicada), recursos a conservar (separando entre tipos de aguas), y bienes y servicios ecosistémicos afectados, así como las conexiones entre componentes y la dirección del flujo (flechas) (**Fig. 3**).

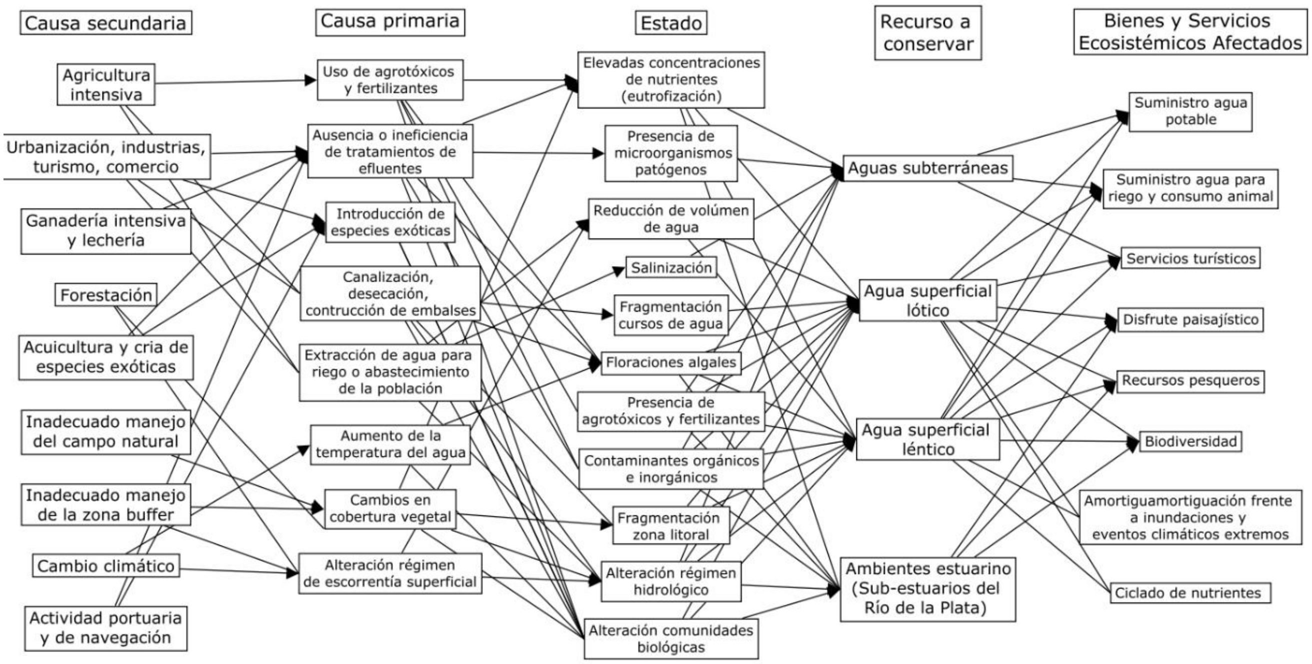


Figura 3. Diagrama de flujo de sistemas acuáticos continentales de Uruguay. De: "Síntesis de la revisión de antecedentes sobre el estado de los ecosistemas y la biodiversidad en Uruguay y las causas de su degradación". Plan Ambiental Nacional para el Desarrollo Sostenible 2018, Convenio CURE-DINAMA.

A partir del diagrama vemos como un inadecuado manejo de las zonas buffers puede generar alteraciones en la cobertura vegetal natural y en el régimen de escorrentía superficial. Esto tiene como consecuencia que haya fragmentación de la zona litoral, aceleración de la eutrofización, alteración del régimen hidrológico y de los volúmenes de agua en sistemas superficiales, así como efectos sobre las comunidades biológicas tanto del ambiente terrestre como acuático. Estas presiones a su vez coocurren con el cambio climático, que potencialmente magnifica algunos de los efectos descritos a través de, principalmente, un aumento de la temperatura y de las precipitaciones. La revisión original incluyó todas las problemáticas descritas en el diagrama de flujo, pero a efectos de la tesis se presenta (de forma resumida) únicamente la información generada a nivel nacional en relación directa o indirecta a las zonas buffer. Esta temática es aún incipiente y poco desarrollada a nivel nacional, pero parece estar creciendo de manera rápida y tomando mayor protagonismo (lo que se evidencia por la publicación reciente de muchos de los estudios).

Como se mencionó, en la actualidad en el Uruguay, los cambios en el uso del suelo generan preocupación respecto a su impacto sobre la calidad del agua dulce y se cuenta con numerosas evidencias de sistemas ya degradados (ej. Bonilla *et al.,* 2015; Goyenola *et al.,* 2015; Barreto *et al.,* 2017; Rodríguez-Gallego *et al.,* 2017; Torremorell *et al.,* 2021; entre otros). Los cambios en la cobertura natural generan frecuentemente una creciente degradación de las zonas litorales adyacentes a los cuerpos de agua (zonas buffer), que son de gran importancia para el mantenimiento de la calidad del agua ya que actúan como filtro de sustancias y materiales particulados y disueltos que llegan desde la cuenca (Lescano *et al.,* 2017; Rodríguez-Gallego *et al.,* 2017; Zarza *et al.,* 2018; Calvo *et al.,* en revisión). A pesar de ocupar generalmente una proporción baja del área total de la cuenca, los bosques riparios concentran niveles muy altos de provisión de servicios ecosistémicos (Nin *et al.,* 2016). La conservación de las zonas buffers en zonas agrícolas, sumada a la aplicación generalizada y eficiente de buenas prácticas de manejo en el uso de fertilizantes, es fundamental para reducir el ingreso de partículas de suelo y nutrientes particulados y disueltos a los cuerpos de agua y/o mitigar su impacto (Arocena *et al.,* 2018; Díaz *et al.,* 2021). A nivel de cuenca, la proporción del terreno con bosque (combinando nativo y plantaciones forestales) se correlaciona de manera inversa con el fósforo en el agua, evidenciando una absorción eficiente del fósforo biodisponible, mientras que existe una fuerte correlación positiva entre el fósforo en agua y el área de uso de suelo agrícola (Gorgoglione *et al.,* 2020). Por su parte, el pastizal natural retiene nutrientes siendo transportados por el agua de escorrentía proveniente de áreas agrícolas, registrando valores de retención de fosfato de 55% y de nitrógeno inorgánico disuelto de 44% (Lescano *et al.,* 2017). Además de nutrientes en exceso, se pueden encontrar en las aguas superficiales otros compuestos cuyo transporte podría ser potencialmente mitigado en presencia de vegetación riparia, tales como residuos de agroquímicos, principalmente de pesticidas (Zaldúa *et al.,* 2010; Nardo *et al.,* 2015; Teixeira de Mello & Stábile, 2016; Williman *et al.,* 2017; Stábile, 2018), así como otras sustancias orgánicas e inorgánicas de origen agropecuario (Arocena *et al.,* 2008; JET-DINAMA, 2010; Griffero *et al.,* 2018) y residuos sólidos. Un estudio reciente en el Río Negro y Río Uruguay encontró residuos de un gran número de pesticidas en los tejidos del 96% de los peces analizados (Ernst *et al.,* 2018), indicando una alta presencia de estas sustancias en los cursos de agua. En centros poblados, por su parte, las márgenes de los ríos son usualmente ocupadas por viviendas u otras infraestructuras, teniendo como resultado, además de la degradación de la vegetación y las márgenes de los cursos de agua (con la consecuente pérdida de su capacidad de purificación), un gran aporte de residuos sólidos y líquidos hacia los cuerpos de agua y un avance sobre la planicie de los sistemas que genera situaciones de riesgo de inundación frecuentes (Piperno *et al.,* 2017; Goñi-Mazzitelli *et al.,* 2019; Freitas *et al.,* 2021). Además, se han registrado niveles altos de contaminación por coliformes y nutrientes, así como valores bajos de oxígeno en agua, pudiendo implicar un riesgo para la salud humana y la integridad de los ambientes urbanos (Alvareda *et al.,* 2021). En estudios nacionales recientes sobre planeamiento urbano se ha comenzado a resaltar la importancia de priorizar la conservación de áreas riparias y humedales, como zonas de amortiguamiento para arroyos y áreas donde la biodiversidad nativa pueda regenerarse (Apud *et al.* 2020).

La eutrofización, una de las principales problemáticas ambientales para el agua dulce, impacta negativamente sobre la integridad de los ecosistemas, degradando como consecuencia la calidad del agua y alterando la configuración natural de las comunidades biológicas (Conde *et al.,* 2002, Mazzeo *et al.,* 2002, Pacheco *et al.,* 2012, Chalar *et al.,* 2013, Benejam *et al.,* 2016; Goyenola *et al.,* 2021), lo que limita en última instancia los posibles usos que pueden dársele a estos sistemas.

Existen cuencas clave a nivel nacional, donde el deterioro de la calidad de agua está ampliamente documentado, como son las cuencas del Río Santa Lucía, Río Negro y Río Cuareim, y las Laguna del Sauce y del Cisne (Bonilla & Meerhoff, 2013; OPP, 2017). La protección y recuperación de las zonas riparias es una de las medidas planteadas en algunas de ellas para revertir el deterioro de la calidad de agua observado en los últimos años. Ejemplos claros a nivel local son la cuenca hidrográfica del Río Santa Lucía y de la Laguna del Sauce ("Plan de acción para la protección de la calidad ambiental y las fuentes disponibles para agua potable en la cuenca del Santa Lucía, de 2013" y "Plan de Acción para la protección de la calidad ambiental y la disponibilidad como fuente de agua potable de la cuenca hidrológica de la Laguna del Sauce", ambos del Ministerio de Vivienda, Ordenamiento Territorial y Medio Ambiente). En sinergia con las medidas de manejo, herramientas recientes de modelado aplicadas a nivel nacional pueden contribuir en un mejor planeamiento de las trasformaciones de usos del suelo en paisajes productivos (López-Pérez, 2018; Rodríguez-Gallego *et al.,* 2019), incluso usando set de datos caracterizados por un alto porcentaje de datos faltantes (Rodríguez *et al.,* 2021).

Además del efecto directo sobre el ingreso de sustancias a los cuerpos de agua, el cambio en el uso del suelo (OPP, 2017) genera alteraciones en el régimen de escorrentía por modificaciones de la cobertura vegetal (Tiscornia *et al.,* 2014). Las características físico-químicas del suelo, fuertemente influenciadas por el uso de la tierra, son un factor importante en el proceso de infiltración, y la consecuente reducción de la escorrentía, generalmente maximizada en áreas buffer (al comparar con cuberturas agrícolas) (Calvo *et al.,* en revisión). El volumen de agua de escorrentía en cultivos, y por ende el transporte de nutrientes, se ven afectados por el sistema de laboreo, el tipo de siembra y de fertilización, y manejos que favorezca una mayor proporción de suelo cubierto podrían disminuir las pérdidas de nitrógeno y fósforo total con el agua de escurrimiento (Castagna *et al.,* 2022). En el caso de la forestación en particular, que ha crecido rápidamente en los últimos 30 años a nivel nacional (Vihervaara *et al.,* 2012), existe una mayor pérdida de agua por evapotranspiración y una disminución en el régimen de escorrentía de alrededor del 20% en años con lluvias promedio (Silveira & Alonso, 2009) y que puede llegar a disminuir la descarga de las cuencas hidrográficas hasta un 30% en períodos de escasas precipitaciones (Silveira *et al.,* 2016).

La reducción y fragmentación de las zonas buffers, producto del rápido aumento de usos productivos del suelo adyacente al bosque nativo, generalmente de pastizales a monocultivos o plantaciones de madera, crean barreras a la fauna y comprometen su conservación (Céspedes-Payret *et al.,* 2009; Andrade-Núñez& Aide, 2010; Ramírez & Säumel, 2022a; Ramírez & Säumel, 2022b). Asimismo, como otros ambientes, las zonas riparias son susceptibles a ser invadidas por especies exóticas, que a largo plazo pueden ver reducida su diversidad y modificar procesos clave del ecosistema (Sosa *et al.,* 2018).

La vegetación riparia podría tener un rol fundamental en modular los efectos asociados a un mayor ingreso de nutrientes, luz y agua de escorrentía a los sistemas dulceacuícolas, como consecuencias directas e indirectas del cambio climático. Los cuerpos de agua dulce son muy vulnerables al cambio climático, que provoca un aumento en la temperatura del agua y cambios en el régimen hidrológico con alteración de las precipitaciones y escorrentía (Bidegain *et al.,* 2012; Aznarez *et al.,* 2021). Se espera que ocurran cambios a nivel de cuencas y a nivel de los ecosistemas, tanto cambios físicos y químicos como en las comunidades de organismos (Meerhoff *et al.,* 2007; González-Bergonzoni *et al.,* 2016; Iglesias *et al.,* 2016) y en particular la pérdida de las especies menos tolerantes a los cambios externos. El aumento de la temperatura ambiente y los cambios en el régimen de precipitaciones pueden actuar de manera sinérgica con el aumento de nutrientes (Meerhoff *et al.,* 2012; 2022), promoviendo aún más las floraciones algales y de cianobacterias (a nivel nacional Haakonsson *et al.,* 2017). Lescano y colaboradores (2017) encontraron una amortiguación menor frente a eventos de alta precipitación, donde los pastizales pasaron de sumidero a fuente de nutrientes.

La variabilidad climática, al modificar la disponibilidad de agua y la temperatura ambiente, puede afectar el crecimiento de los bosques nativos y su productividad, con alteraciones potenciales sobre el secuestro de carbono y la conservación de la vida silvestre (Lucas *et al.,* 2017; Lucas *et al.,* 2018). Asimismo, la conversión de la tierra de pastizales a cultivos puede provocar cambios en el balance de carbono, ya que las emisiones de CO_2 de los pastizales se ha encontrado que son casi neutras, mientras que en los cultivos las emisiones son varias veces mayores (Castaño-Sánchez *et al.,* 2021), contribuyendo al aumento de gases de efecto invernadero (GEI) en la atmósfera.

CAPÍTULO 2. RETENCIÓN DE NUTRIENTES EN LA ESCORRENTÍA SUPERFICIAL Y SUBSUPERFICIAL EN ZONAS BUFFER DE PASTIZAL, ARBUSTAL Y BOSQUE

Este capítulo resume la aproximación general, objetivos y principales resultados desarrollados en el Articulo 1 "Agricultural surface and subsurface runoff and nutrient retention by grassland, shrubland, and tree-dominated buffer zones".

Bajo un escenario de cambios en el uso de la tierra y climáticos, y debido a su estrecha dependencia con características específicas de cada sitio, se necesita una comprensión más precisa del movimiento del agua de escorrentía a través de la superficie y subsuperficie del suelo, tanto para coberturas agrícolas como naturales. Para enfrentar la crisis de degradación de la calidad del agua del principal reservorio de agua potable (Embalse de Paso Severino) se tomó la medida de alambrar y proteger el área buffer, prohibiendo allí cambio de la cobertura vegetal natural, los cultivos, la ganadería y la aplicación de agroquímicos, con el fin de favorecer el crecimiento y regeneración de la vegetación natural en los márgenes y minimizar, a largo plazo, el aporte de nutrientes y sedimentos transportados por la escorrentía y la erosión en el embalse. Nuestro estudio tuvo como objetivo evaluar la capacidad de retención de nutrientes, luego del alambrado y protección de la zona riparia. Para ello realizamos un seguimiento *in situ* dentro del periodo de un año de las aguas superficiales y subsuperficiales de tres zonas buffers y de los cultivos adyacentes situados terreno arriba (praderas mejoradas), para comprobar su capacidad de retención de nutrientes bajo un amplio rango de condiciones naturales de precipitación (**Fig. 4**).

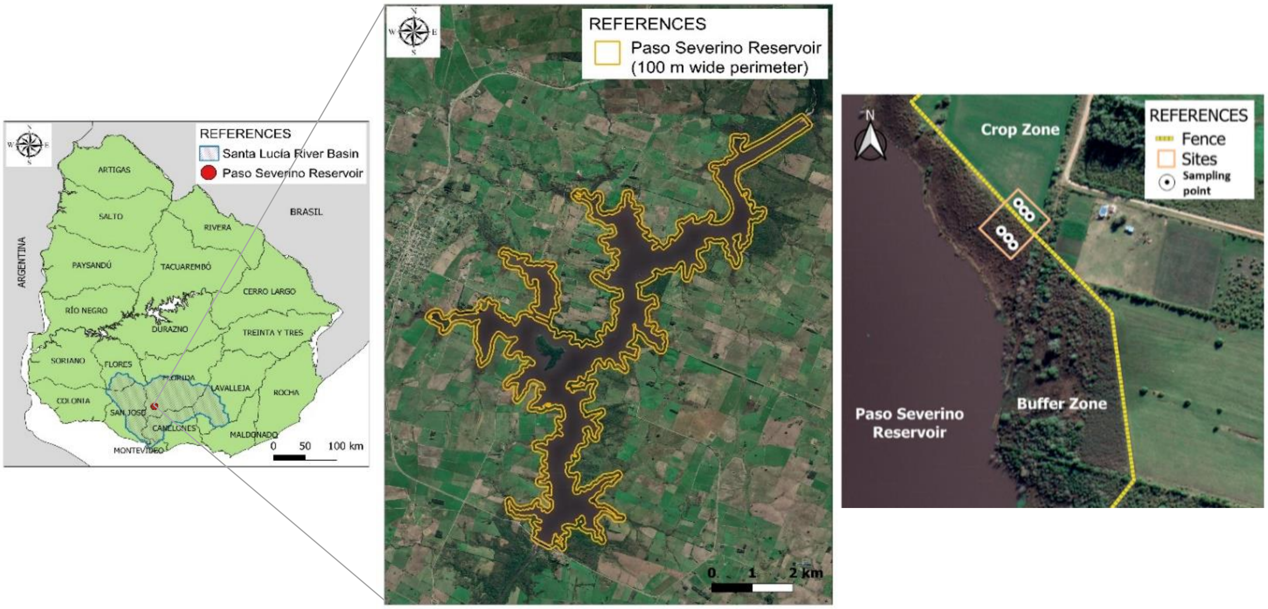


Figura 4. Mapa mostrando la ubicación del área de estudio y su cuenca, así como el perímetro cercado alrededor del Embalse (en amarillo) y un ejemplo de la ubicación de un sitio con sus puntos de muestreo en el cultivo y zona buffer, a un lado y otro del cerco de restricción. **Artículo 1.**

Para el estudio se seleccionaron tres áreas buffers de 100 m de ancho ubicadas al borde del embalse y dentro del área cercada: 1. una con pastizal, 2. una con arbustos (pastizal arbustizado) y 3. una con bosque nativo, todas ellas con agricultura pendiente arriba, fuera de la zona de exclusión (pasturas artificiales). Se analizaron varias características del suelo, relevantes para comprender la movilidad potencial del fósforo y el nitrógeno en la escorrentía superficial y subsuperficial, así como una caracterización de la vegetación en cada sitio basada en formas de vida y biomasa. La escorrentía superficial se generó artificialmente usando un mini-simulador. Las simulaciones de escorrentía se realizaron dentro de las 24 a 36 horas posteriores a los eventos de lluvia seleccionados (n eventos=4). Por su parte, el agua subsuperficial se colectó en dispositivos de diseño propio enterrados en el suelo con una sección perforada ubicadas a la altura del horizonte B del suelo (n eventos=8) (metodología desarrollada en capítulo 2).

Encontramos una fuerte evidencia de retención de nutrientes por zonas buffer y la consiguiente mejora de la calidad del agua de escorrentía agrícola. Los tres tipos de cobertura vegetal (es decir, pastizal, arbustal y bosque) redujeron la cantidad de agua que llegaba al embalse y, por lo tanto redujeron, en distintas proporciones, las cargas de nutrientes disueltos y particulados. Entre los tres, la cubierta forestal mejoró la retención de fósforo, mientras que los pastizales fueron el regulador más fuerte de la dinámica del nitrógeno. La proporción de PO_4 en PT (µg/L) fue en general alta, con una media global de 70% y con una tendencia a ser superior en los cultivos. En las zonas buffer, la carga de todos los nutrientes (PT, PO_4, NT, NO_3 y NH_4) aumentó linealmente con valores más altos de tasa de escorrentía.

Nuestros resultados sugieren que grandes cantidades de fósforo de los cultivos se estarían movilizando al cuerpo de agua a través del agua de escorrentía, la mayor parte en forma disuelta, y que las zonas buffer desempeñan un papel fundamental en la retención de las mismas. Las áreas buffers compuestas por una combinación de vegetación herbácea y leñosa alcanzarían la tasa máxima de retención de nutrientes, cuando se considera al fósforo y nitrógeno de manera simultánea. Asimismo, nuestros resultados mostraron que en las zonas buffer, independientemente del tipo predominante de vegetación, el transporte superficial tanto de P como de N aumentó con el aumento de la tasa de escorrentía. En área de estudio se prevén eventos de lluvias extremas más frecuentes, esto podría ser particularmente relevante y probablemente afecte la retención de nutrientes por parte de la vegetación riparia. A pesar de sus limitaciones, los resultados muestran que las medidas de gestión disminuyen la entrada de nutrientes al embalse y contribuyen a cumplir los objetivos de calidad del agua, pero podría reducirse la capacidad buffer en un futuro escenario de cambio climático. Medidas tomadas a nivel de toda la cuenca y en el contexto del cambio climático son esenciales (Nobre *et al.,* 2020). Este conocimiento es crucial para anticipar mejor la influencia del cambio climático global sobre fuentes difusas de nutrientes.

CAPÍTULO 3. METODOLOGÍA PARA EL MONITOREO SUBSUPERFICIAL DE APORTES DE NUTRIENTES A SISTEMAS HÍDRICOS, EMBALSE DE PASO SEVERINO, URUGUAY.

Este capítulo resume la aproximación general, objetivos y principales resultados desarrollados en el Articulo 2 “Metodología para el monitoreo subsuperficial de aportes de nutrientes a sistemas hídricos, embalse de Paso Severino, Uruguay”.

El monitoreo del agua subsuperficial es complejo debido a que su movimiento no es uniforme y las técnicas apropiadas para su detección y caracterización no son sencillas. El objetivo de este trabajo fue describir el diseño y los resultados principales de dispositivos experimentales usados para captar, almacenar y caracterizar las propiedades físico-químicas del agua subsuperficial del suelo a una profundidad específica, proveniente de sitios con distintos usos.

El diseño de los dispositivos resultó ser efectivo en la colecta de agua subsuperficial en distintas coberturas (**Fig. 5**). Los colectores fueron de simple construcción y de bajo costo, fáciles de trasladar, y con un tiempo de construcción e instalación de cada dispositivo relativamente breve. Los materiales utilizados fueron de uso doméstico frecuente y por lo tanto muy accesibles logística y económicamente. La profundidad a la que se enterró el tubo y en la que se ubicó la sección con perforaciones se determinó según las características del suelo, buscando representar el horizonte B. Los colectores se dispusieron en transectas perpendiculares a la línea de costa del embalse. En cada uno de los sitios se instalaron tres colectores en el predio con cultivos y tres en las zonas buffer (i.e. pastizal, arbustal y bosque nativo).

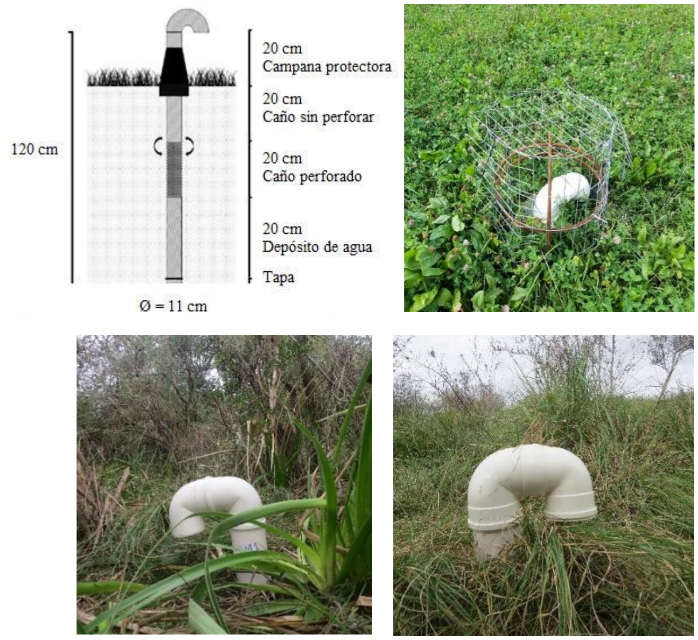


Figura 5. Esquema del diseño de los colectores de agua con el detalle de las dimensiones de cada tramo, e imágenes de colectores instalado dentro de un predio cultivado y en distintas zonas buffers. **Artículo 2**.

Todos los colectores juntaron agua, aunque con variabilidad entre colectores adyacentes, en especial cuando las precipitaciones fueron bajas. La extracción del agua almacenada resultó efectiva y sencilla, siempre y cuando la precipitación fuera suficiente para colectar volumen. Los dispositivos diseñados permitieron una colecta eficiente y precisa de agua subsuperficial, lo que permitió una normal determinación de distintos parámetros físico- químicos y de nutrientes (nitrógeno y fósforo) disueltos. Asimismo, fue posible la estimación simultánea de concentración y carga de nutrientes en el agua subsuperficial del suelo, aunque se precisan correcciones para mejorar las estimaciones. El uso generalizado de estos dispositivos parece muy promisorio, haciendo ajustes de acuerdo a la estructura y profundidad del horizonte de interés y también en función de la porosidad y granulometría del suelo, pudiendo ser necesario ajustar la densidad de los poros y la capacidad de almacenamiento. En futuros diseños se deberá, aumentar la longitud del tubo para aumentar la capacidad de almacenamiento, o tener un sistema de extracción y cuantificación del agua excedente para estimar con precisión el volumen circulante. El colector diseñado es un dispositivo útil para entender mejor los procesos hidrológicos y biogeoquímicos, permitiendo recolectar agua en pasos secuenciados en el tiempo.

CAPITULO 4. LIBERACIÓN DE NUTRIENTES Y FLUJOS DE CARBONO EN CULTIVOS Y SUELOS BUFFER BAJO REGÍMENES DE LLUVIA Y TEMPERATURA AMBIENTE CONTRASTANTES

Este capítulo resume la aproximación general, objetivos y principales resultados desarrollados en el borrador del Articulo 3 "Experimental warming and rainfall regimes lead to higher nutrient release and changes in Carbon fluxes from crop and buffer zone soils".

Bajo un escenario de cambio climático, se espera que las temperaturas medias aumenten y se magnifiquen los efectos de las inundaciones extremas, que a su vez serán más frecuentes. Esto podría conducir a mayores tasas de liberación de nutrientes desde los suelos inundados, y a medida que aumenta la temperatura, mayores tasas de movilización, así como efectos sobre las emisiones netas de CO_2 del suelo. Comprender los mecanismos biogeoquímicos afectando el transporte de nutrientes en las zonas riparias ayudará a predecir la efectividad en la remoción de nutrientes bajo diferentes escenarios futuros, así como la ocurrencia de potenciales retroalimentaciones con el cambio climático. En este estudio imitamos experimentalmente ambientes riparios y agrícolas saturados de agua e inundados, simulando un escenario de lluvia ligera de dos semanas y un escenario de lluvia intensa de corto plazo, en combinación con un escenario de calentamiento, sobre suelos de cultivos y bosques de una cuenca hidrográfica clave en Uruguay (Paso Severino).

El diseño fue completamente factorial con 32 microcosmos experimentales en total: 2 coberturas del suelo (cultivo y bosque), 2 temperaturas (control 17.5 °C y elevada 20.5 °C) y 2 regímenes de lluvia: una lluvia ligera de larga duración (LR) y una lluvia ligera de larga duración + lluvia fuerte de corta duración (HR), con 4 repeticiones de cada tratamiento y una duración total de 23 días (**Fig. 6**). Después de una fase inicial de aclimatación, el día 5 comenzamos la lluvia ligera simulada en todos los tubos, agregando agua destilada de manera cuidadosa y gradual hasta la superficie del suelo. En el tratamiento de lluvia ligera (LR), el suelo permaneció anegado durante 15 días, simulando un período largo y sostenido de lluvia ligera. Mientras tanto, en el tratamiento de lluvia ligera + fuerte (HR), el día 9 inundamos los tubos con agua destilada aproximadamente 10 cm sobre la superficie del suelo. Los tubos permanecieron inundados durante 5 días, simulando un episodio de lluvia intensa. Luego, el agua sobre el suelo se eliminó cuidadosamente con una jeringa y el suelo permaneció anegado durante 5 días más, y 4 días más sin recibir agua (fase de recuperación) hasta el final del experimento. Sacamos muestras del agua intersticial del suelo cada 3-4 días desde una profundidad intermedia de la columna para analizar fosfato. El agua de inundación se muestreó todos los días durante el evento de lluvia extrema y se analizó fósforo total, fosfato, nitrógeno total, nitrato y amonio, Durante todo el experimento cada 2-3 días hicimos mediciones de CO_2 a partir de los cuales estimamos los flujos de difusión.

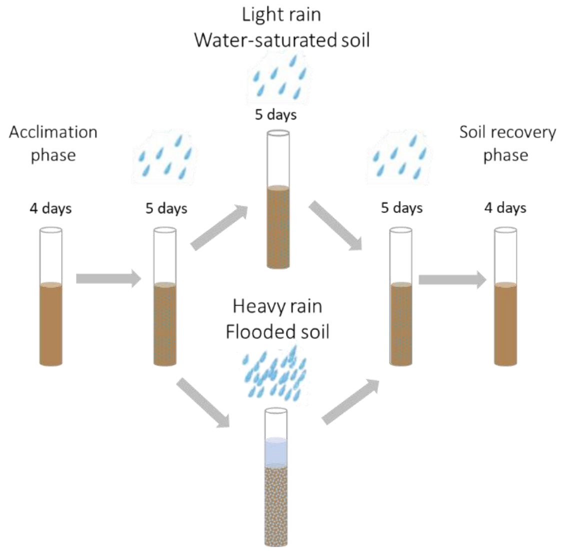


Figura 6. Diseño esquemático del experimento y la duración (en días) de los períodos: aclimatación, lluvia ligera, lluvia fuerte y recuperación.

Nuestros resultados sugieren que la saturación del suelo y las inundaciones movilizan fósforo y pueden potencialmente aumentar las emisiones de CO_2, y un posible efecto sinérgico del calentamiento. Sin embargo, no podemos generalizar sobre los resultados de la variabilidad climática en los regímenes de temperatura y lluvia, ya que dependen en gran medida del uso del suelo. Si el suelo de cultivo está anegado o inundado, se produce una exportación significativa de PO_4, y el calentamiento actúa intensificando la liberación. En los suelos de los bosques, la liberación ocurrió solo en el caso extremo de inundación y con un efecto de calentamiento más moderado. Para el nitrógeno, las cargas movilizadas durante la inundación de corto plazo no fueron altas para los estándares de calidad del agua, pero detectamos cambios en las proporciones de nitrato y amonio. Para el nitrógeno total y el nitrato, registramos una mayor liberación general con el calentamiento y desde el suelo del bosque, mientras que la liberación de amonio fue mayor en el bosque, pero no mostró un efecto evidente debido al calentamiento. Registramos un cambio de sumidero a fuente de CO_2 en suelos forestales bajo condiciones de calentamiento y re-humedecimiento, sugiriendo que el calentamiento global acelerará la descomposición del carbono orgánico del suelo y, potencialmente, generará una retroalimentación positiva sobre el aumento de temperatura

Si el área inundada es principalmente agrícola, podría haber una elevada tasa de liberación de fosfato a un cuerpo de agua cercano, con una liberación aun mayor bajo calentamiento. Esto tiene una relevancia importante para el manejo de los ecosistemas terrestres y acuáticos, ya que esta forma es asimilada directamente por el fitoplancton (microalgas y cianobacterias) y las plantas acuáticas. En eventos de lluvia de gran magnitud cuando el suelo se inunda, los beneficios de una zona riparia no fueron tan evidentes, ya que la concentración de fósforo en el agua inundada del bosque fue similar a la del suelo de cultivo y el nitrógeno fue generalmente más alto en el bosque. Los hallazgos son importantes para comprender y predecir los efectos de las inundaciones, que actúan simultáneamente con el calentamiento y el cambio de uso del suelo. A su vez, se espera que contribuyan a la generación de modelos que permitan predecir mejor el transporte de P y N en los ambientes riparios, así como los flujos de C.

CAPITULO 5. LOS PULSOS DE FLUJO AFECTAN AL PERIFITON DE MANERA DIFERENTE SEGÚN LAS CONDICIONES LOCALES DE LUZ Y NUTRIENTES EN ARROYOS EXPERIMENTALES

Este capítulo resume la aproximación general, objetivos y principales resultados desarrollados en el Articulo 4 "Flow pulses shape periphyton differently according to local light and nutrient conditions in experimental lowland streams".

Alteraciones repentinas en las descargas pueden tener grandes efectos en el funcionamiento ecológico y la biodiversidad de los arroyos. Se espera que los eventos de precipitación aumenten en magnitud y frecuencia en muchas regiones a medida que cambia el clima y en combinación con cambios en el uso del suelo que disminuyen la infiltración, la frecuencia y la magnitud de los eventos repentinos de caudal alto probablemente aumentarán como consecuencia. Asimismo, la expansión de la superficie de cultivo en detrimento de las cubiertas vegetales naturales afecta la disponibilidad de nutrientes y luz en los sistemas acuáticos. En el artículo 4, nuestro objetivo fue estudiar experimentalmente los efectos de pulsos de flujo repentinos sobre la comunidad de perifiton, imitando eventos de precipitación extrema en arroyos. Se prevé que eventos extremos ocurran con mayor frecuencia con el cambio climático en algunas regiones, y que la sustitución de zonas buffer por usos productivos maximice la escorrentía ingresando a los cuerpos de agua, así como el ingreso de nutrientes y la disponibilidad de luz.

El experimento se realizó en ocho canales al aire libre, alimentados de agua sin filtrar bombeada desde un arroyo cercano, con un flujo unidireccional continuo y sin recirculación (**Fig. 7**). Inicialmente hubo una fase pre-experimental en la que se ajustaron los ocho canales para generar un gradiente de flujo basal continuo. Usamos sustratos de difusión de nutrientes para generar dos concentraciones: Control (sin adición de nutrientes) y NP (enriquecido, simulando un escenario de eutrofización). Los sustratos fueron asignados a cada uno de los canales y dentro de los canales a zonas con 50% de reducción de luz (Sombra) o sin cobertura, totalmente expuestos a la luz natural (Luz). Después de la fase de colonización (1 mes), removimos de los canales la mitad de los sustratos y los sustratos restantes se expusieron a un aumento abrupto de flujo (proporcional al flujo basal) durante 3 días, como potencial factor de perturbación, hasta que nuevamente se reanudaron los valores de flujo basales iniciales y permanecieron así por 4 días más. De los sustratos de ambas fases (pre y post pulso) analizamos clorofila *a* y composición taxonómica, clasificando los organismos según el tipo y forma de vida.

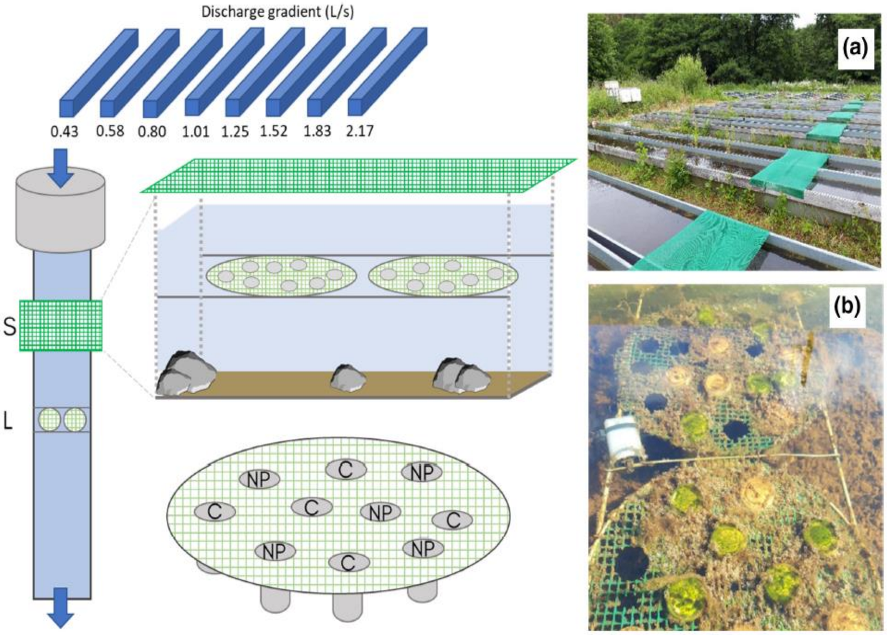


Figura 7. Diseño experimental que incluyó ocho canales exteriores de 12 m de largo (a) con un gradiente de flujo basal continuo (0.43, 0.58, 0.80, 1.01, 1.25, 1.52, 1.83 y 2.17 L/s). Los sustratos de difusión de nutrientes (SDN) para la colonización de perifiton, con y sin enriquecimiento de nutrientes (C/NP), se colocaron en cada canal en secciones sombreadas (S) o con acceso total a la luz natural (L). Las imágenes muestran los canales y los sustratos después de 4 semanas de colonización. **Artículo 4**.

En general, encontramos que la disponibilidad de luz aumentó significativamente el crecimiento del perifiton, mientras que el enriquecimiento de nutrientes solo lo hizo cuando se combinó con el acceso total a la luz, tanto antes como después de los pulsos de flujo. Un mayor flujo estimuló el crecimiento del perifiton antes del pulso de flujo, pero solo cuando se combinó con pleno acceso a la luz. Con respecto al pulso, encontramos respuestas contrastantes en el crecimiento de perifiton dependiendo de las condiciones de flujo basal: en los más bajos, el pulso promovió el crecimiento del perifiton, mientras que en los flujos más altos hubo una pérdida de biomasa. El efecto positivo máximo del pulso sobre el aumento de biomasa ocurrió en el perifiton expuesto al flujo basal más bajo con enriquecimiento de nutrientes y pleno acceso a la luz. La riqueza taxonómica fue mayor después del pulso para todos los flujos base, excepto para las condiciones de flujo más bajas. La composición del perifiton, en términos de biovolumen de cada género encontrado, cambió luego de la perturbación generada por el pulso y también difirió entre el control y el tratamiento de enriquecimiento de nutrientes. La comunidad de perifiton mostró baja resistencia contra el pulso independientemente del flujo basal inicial, pero disminuyendo progresivamente con el aumento del flujo.

En nuestro experimento demostramos que los efectos de pulsos de flujo sobre el perifiton tienen efectos no lineales en términos de biomasa, como era de esperar, pero también que los efectos pueden variar fuertemente en cuanto a la composición dependiendo de la estructura de la comunidad inicial, que a su vez está mediada por el caudal basal, y por la disponibilidad de luz y nutrientes. El pulso de flujo promovió una mayor riqueza taxonómica, lo que sugiere un reinicio parcial de la sucesión de las comunidades y nuevas oportunidades para los colonizadores. Las perturbaciones del flujo favorecen diferencialmente géneros distintos, sin embargo, la relación entre el flujo basal y la riqueza no fue lineal. Independientemente del flujo y los recursos, las comunidades de perifiton mostraron baja resistencia ecológica frente al pulso con cambios en clorofila *a*, biovolumen y riqueza taxonómica.

La disponibilidad de luz aumentó significativamente la resistencia de la comunidad, lo que respalda su papel como principal impulsor del incremento de biomasa. En un escenario de aumento de luz que llega a los arroyos, como resultado de la deforestación o la pérdida de vegetación riparia u otros cambios en el uso del suelo, nuestros resultados sugieren un mayor crecimiento del perifiton y una mayor resistencia contra el control ejercido naturalmente por los pulsos de flujo sobre la biomasa. Nuestros resultados destacan la importancia de estudiar simultáneamente la dinámica temporal de múltiples estresores. Los entornos ambientales locales, condicionados en gran parte por la vegetación riparia, van a determinar en última instancia los efectos netos de los eventos extremos de flujo en la estructura de la comunidad de perifiton y probablemente también en varios procesos ecosistémicos.

CONSIDERACIONES FINALES

La vegetación riparia o buffer cumple un rol ecosistémico invaluable como moderador de procesos que conectan las actividades que se llevan a cabo a nivel de cuenca con los ecosistemas acuáticos. Dichos procesos y sus funciones asociadas responden a estresores climáticos y de uso del suelo, lo que afecta su dinámica y provoca cambios en los mecanismos que actúan en la interfase terrestre-acuática, con consecuencias sobre los ecosistemas de agua dulce. Las zonas buffer se ven afectadas por la sostenida presión sobre los ecosistemas naturales, producto del crecimiento de la población humana y la intensificación de diversas actividades socio-económicas, y Uruguay no escapa a estas problemáticas. A pesar de los avances recientes, el conocimiento generado a nivel local es aún muy escaso.

La exportación de nutrientes desde áreas agrícolas puede ser un fuerte regulador del estado de la calidad del agua dulce (Foley *et al.,* 2005, Bender *et al.,* 2018). En el embalse de Paso Severino se están movilizando grandes cargas de fósforo desde los cultivos y hacia el agua, la mayor parte en forma disuelta y a través del agua de escorrentía superficial y subsuperficial. Frente a este escenario y a la actual intensificación productiva, las zonas riparias están teniendo un rol fundamental en la reducción de dicho transporte por la cuenca. Coincidiendo con trabajos previos (ej. Cao *et al.,* 2019), nuestros resultados sugieren que las áreas buffer compuestas por una combinación de vegetación herbácea y leñosa alcanzarían la máxima tasa de retención de nutrientes, considerando tanto la escorrentía superficial como el flujo moviéndose sub-superficialmente por el suelo.

En los cultivos analizados encontramos suelos degradados de acuerdo a sus parámetros físico-químicos y con altas concentraciones de nutrientes, principalmente en las capas más superficiales. Esto ocurre probablemente por acumulación gradual por aplicación de fertilizantes (Sharpley *et al.,* 2000, Tiessen *et al.,* 2010). Además, encontramos una infiltración muy reducida en comparación con suelos buffer, que en consecuencia afecta la escorrentía y provoca cambios en el ciclo hidrológico (Gordon *et al.,* 2008; Boizard *et al.,* 2013; Alaoui *et al.,* 2018). Generamos evidencia que apoya la idea de que las diversas presiones que reciben actualmente las zonas buffer podrían estar comprometiendo los mecanismos mediante los cuales aportan resistencia y resiliencia a los ecosistemas acuáticos. Mas allá de la remoción de las coberturas vegetales naturales, o la reducción de su área al expandirse las actividades productivas, la intensificación del sistema agrario provoca que más nutrientes se transporten pendiente abajo por la cuenca. Frente al movimiento de cargas altas y consecuente enriquecimiento del suelo (Goyette *et al.,* 2018), existe el riesgo potencial de que por efecto de ciertas forzantes climáticas (variabilidad en la temperatura y precipitaciones) las zonas buffer se conviertan en fuente de nutrientes. Por ello, una gestión eficaz de la calidad del agua requiere, además de la consideración del uso de la tierra en toda la cuenca, que se haga en el contexto del cambio climático (Nobre *et al.,* 2020).

Nuestros resultados sugieren que la eficiencia en la retención de nutrientes de los suelos con cobertura buffer podrían estar menos sujetos a las variaciones climáticas que aquellos con cultivos. Sin embargo, en zonas riparias encontramos mayores cargas de fósforo y nitrógeno (tanto de sus formas totales como disueltas) a mayor tasa de escorrentía superficial, independientemente del tipo de vegetación dominante. Los modelos climáticos predicen para Uruguay un aumento en la precipitación anual y en la intensidad y frecuencia de eventos de lluvias extremas (Marengo *et al.,* 2010; 2017). Dicha respuesta podría ser particularmente relevante y potencialmente disminuir la eficiencia en la remoción de las zonas de amortiguamiento, como se ha sugerido en estudios previos (Bu *et al.,* 2016). Asimismo, a partir de este estudio, encontramos que en un escenario de inundación del bosque ripario se movilizarían altas cargas de P hacia el agua, probablemente debido a su rápida disponibilidad en el suelo, disminuyendo la eficiencia de retención de la zona buffer a largo plazo. Según nuestros resultados, además, una mayor temperatura y disponibilidad de agua promovería un incremento en la emisión de CO_2 hacia la atmósfera desde los suelos terrestres enriquecidos con materia orgánica, como son los bosques. Este aumento sugiere que el calentamiento climático acelerará la descomposición del carbono orgánico del suelo promoviendo una retroalimentación positiva sobre el sistema climático (Giardina *et al.,* 2014; Soong *et al.,* 2021). El anegamiento temporal o la inundación de suelos cultivados llevó sistemáticamente a una mayor movilización de nutrientes al agua que desde suelos buffer, con procesos muy influenciados por el calentamiento que intensificaron los efectos a mayores temperaturas.

Nuestros resultados experimentales sugieren un efecto sinérgico de factores estrechamente vinculados a la presencia o ausencia de vegetación riparia a nivel de comunidades acuáticas (perifiton). Un aumento en la biomasa de perifiton con el aumento del flujo de agua (mayor en ausencia o disminución de vegetación riparia), y el significativo papel de la luz en la regulación de estas respuestas (mayor en ausencia de vegetación riparia), así como los cambios en la composición específica con el incremento de nutrientes (también mayor en ausencia de vegetación riparia), muestran la relevancia de mantener la cobertura riparia, y más aún el dosel en los arroyos de bajo orden. Las zonas riparias podrían contribuir a mitigar un desarrollo exacerbado de perifiton (McDowell *et al.,* 2020) en respuesta a presiones climáticas y de cambio en el uso del suelo, particularmente en escenarios de mayor temperatura y lluvias extremas.

Nuestros resultados sugieren que la medida de gestión a gran escala aplicada en Paso Severino (zona de exclusión buffer) puede potencialmente disminuir el ingreso de nutrientes al embalse y contribuir a cumplir los objetivos de calidad del agua. El desarrollo de estrategias globalmente aplicables para mitigar el impacto negativo de la creciente variabilidad climática es complejo, ya que las respuestas dependen del uso del suelo. De forma paralela y complementaria, la aplicación de buenas prácticas agrícolas resulta fundamental para que las zonas riparias funcionen dentro de los parámetros deseables desde un punto de vista ambiental (Tong *et al.,* 2017). Por otro lado, resulta necesario incorporar nuevas medidas de manejo de la vegetación acumulada, para no contrarrestar los aspectos positivos de las zonas buffer (Cabrera-Lamanna, 2020).

A pesar de ser medidas que generan un conflicto de uso entre diferentes actores por la dificultad de conciliar aspectos de la producción con el cuidado del medioambiente, así como dificultades en el control de su aplicación, regular el uso del suelo en las márgenes de los sistemas y excluir las actividades productivas parece ser clave para ayudar a mejorar el impacto de las actividades agrícolas a nivel de cuenca sobre la calidad del agua (Amuchástegui *et al.,* 2015). El mantenimiento de las zonas riparias, además de tener efectos estrictamente ecológicos, trae beneficios económicos directos. Una gestión sostenible de dicho ecosistema supone un gran ahorro en posteriores inversiones para su restauración y mantenimiento, tanto para sistemas lóticos como lénticos (Xiang *et al.,* 2016), aun cuando el área riparia está muy acotada debido a la intensa actividad agrícola cerca de los cursos de agua (Balestrini *et al.,* 2011). El establecimiento o restablecimiento de vegetación riparia en la zona de fluctuación del nivel del agua parece ser un método efectivo para reducir el ingreso de nutrientes al embalse, tanto por escorrentía superficial y subsuperficial, en situaciones de precipitaciones medias, así como en situaciones de inundación o anegamiento.

PERSPECTIVAS

La gran complejidad y heterogeneidad de las zonas riparias, así como su sensibilidad a las perturbaciones, hacen compleja su regulación, gestión y conservación. Sin embargo, a partir del presente trabajo y los antecedentes internacionales y nacionales, parece incuestionable que las zonas riparias o buffers constituyen un "escudo natural" de los sistemas de agua dulce. Según evidencia previa, las medidas de manejo que buscan reducir los nutrientes una vez ingresados en los cuerpos de agua pueden ser menos eficientes y, a la larga, más costosas que las medidas que apuntan a disminuir los ingresos externos de nutrientes. En estas últimas debería estar el foco y la prioridad a nivel país, siendo las más relevantes la aplicación de buenas prácticas de manejo en los predios productivos y la protección de la vegetación riparia en las márgenes de los sistemas.

El proyecto planteado se espera que contribuyan a generar bases para una gestión más eficaz de los recursos hídricos a nivel de cuenca, en particular de posibles medidas de conservación de las áreas riparias. Los resultados obtenidos pueden contribuir a la toma de decisión en cuanto a la conservación de las zonas riparias existentes, así como en situaciones donde las mismas han sido removidas y deben ser restauradas. Las zonas riparias, por su rol de últimas barreras de protección de los ecosistemas acuáticos, deberían ser consideradas prioridad al momento de diseñar estrategias de conservación. Para ello es necesario la generación de más conocimiento a nivel local, que contribuya a ajustar medidas planteadas para otras regiones según las condiciones locales y los escenarios futuros predichos para la región. Debido a su gran magnitud, es importante hacer un seguimiento de las medidas de restauración implementadas en Paso Severino, para así evaluar efectos a largo plazo y su potencial expansión dentro de la cuenca, o incluso su replicación en otros sistemas deteriorados del país. Asimismo, se debería evaluar la extensión de la medida de protección a afluentes y especialmente a las cabeceras para maximizar la retención, ya que estas áreas pueden llegar a ser muy relevantes para la eliminación de nutrientes a escala de cuenca (Correll, 2005).

Existen otras cuencas clave a nivel nacional, además de la del río Santa Lucía, por su tamaño y usos, en las que coexiste la actividad agropecuaria con importantes centros urbanos (y en algunos casos también industriales). En ellas el deterioro de la calidad de agua está documentado y deberían ser consideradas cuencas prioritarias para la implementación de medidas, como ser la protección del área riparia, con un seguimiento adecuado para asegurar su sostenibilidad en el tiempo.

A partir de los hallazgos, surgen muchos otros aspectos que ameritan más estudios, por ejemplo, sería interesante incluir en el análisis distintas combinaciones de anchos de las coberturas vegetales evaluadas y la inclusión en los experimentos de suelo del análisis de la comunidad microbiana y sus respuestas. Con respecto a la colecta de datos *in situ*, se deben mejorar los métodos de colecta de agua de escorrentía (tanto superficial como subsuperficial), y ajustar los valores de carga estimados, ya que a pesar de su utilidad y aporte al entendimiento de los procesos sucediendo, son potenciales y no necesariamente son las cargas precisas que llegan al Embalse de Paso Severino actualmente. Asimismo, resulta fundamental que las líneas de trabajo futuras continúen en la generación de predicciones de los efectos que tendrán los cambios en el uso de la tierra y climáticos sobre los sistemas dulceacuícolas mediante procesos que involucran a las zonas riparias y determinar la importancia relativa de los mecanismos asociados, así como los vínculos entre las respuestas, la ocurrencia de feedbacks positivos y negativos, etc. Asimismo, es urgente la investigación enfocada a generar y ajustar medidas de mitigación frente a los (muchos) cambios actuando de forma simultánea. Estos resultados sientan las bases para futuros trabajos que analicen respuestas a nivel de redes tróficas completas, más allá de especies o grupos en particular. Por la complejidad y diversidad de temas vinculados al entendimiento del rol ecosistémico de las zonas riparias (del área agronómica, hidrológica, limnológica, biogeoquímica, climática) la multidisciplinariedad en el análisis resulta fundamental.

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PARTICIPACIÓN EN PROYECTOS VINCULADOS A LA TESIS

1. Plan Ambiental Nacional para el Desarrollo Sostenible: Síntesis de la revisión de antecedentes sobre el estado de los ecosistemas y la biodiversidad en Uruguay, y las causas de su degradación. EJE 3: SISTEMAS DE AGUA DULCE. 6 meses.

   Convenio de cooperación técnica CURE-DINAMA.

2. Evaluación de la dinámica del fósforo en zonas de amortiguación del Embalse Paso Severino y propuestas de alternativas de manejo para reducir los aportes difusos de este nutriente. 2 años.

   Convenio de cooperación técnica CURE-DINAMA.

3. Climate change effects on phytoplankton and periphyton in streams and shallow lakes. 6 meses.

   Pasantía en el exterior ANII.

FINANCIACIÓN

2018 - 2020	Beca Posgrado, ANII
2019 -2020	Iniciación a la Investigación, CSIC
2021	Beca de finalización, CAP

ARTICULO 1

Agricultural surface and subsurface runoff and nutrient retention by grassland, shrubland, and tree-dominated buffer zones

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Abstract

Agriculture is the main driver of land-use change worldwide, and its intensification increases eutrophication of surface water bodies among a wide range of environmental, societal, and economic problems. One of the most frequent strategies to reduce the external loads of nutrients is the maintenance or re-establishment of riparian zones. We conducted *in situ* monitoring of surface and subsurface water in three different vegetated buffer zones (grassland, shrubland, forest), and adjacent croplands for an entire year, to test nutrient retention capacity under a range of natural precipitations levels. All three crop areas had soils with lower quality in their physical properties in comparison to their respective buffer zones. Start of runoff was delayed and infiltration was greater in the three buffer zones, compared to crop areas. Surface runoff and TP and PO_4 loads were lower in the three buffers than crop areas, however, nutrient concentrations were lower only in the forest riparian buffer. TP and PO_4 loads in surface runoff in buffer zones increased linearly with runoff rate. Lower NO_3 loads were recorded in buffers, and NO_3 load was higher with grater runoff. In subsurface runoff, PO_4 concentration and load were lower in the three buffer zones than crop areas. PO_4 concentration increased with temperature in crop areas and PO_4 load increased with precipitation. Lowest NO_3 concentrations and loads were observed in the grassland buffer. In both crop and buffer areas, NO_3 concentration and load increased with temperature. Our results suggest that riparian buffers comprised of herbaceous and woody vegetation have highest rates of phosphorus and nitrogen retention. Management of riparian areas has great potential to mitigate eutrophication of water bodies, but future climatic scenarios for the study region forecast greater precipitation, which could reduce the effectiveness of riparian buffers.

Keywords: Nutrient removal, rainfall, climate change, riparian vegetation

Highlights

- We investigated nutrient retention capacity of riparian buffer zones with forest, shrubland or grassland.
- Nutrient retention in both surface and subsurface runoff water was greater in riparian buffer areas than adjacent crop areas.
- Forest cover enhanced P retention, and grassland had the greatest effect on N dynamics.
- Mixed herbaceous and woody riparian buffers should yield greatest nutrient retention.
- Projected increase in regional precipitation may reduce the capacity for nutrient retention in riparian buffer zones.

1. Introduction

Agriculture is the main driver of land use change worldwide, and its intensification increase the diffuse transfer of nutrients to surface water bodies causing a wide range of environmental, societal, and economic problems (Foley *et al.* 2005, Bender *et al.* 2018). Eutrophication induced by agricultural runoff is one of the main causes of aquatic ecosystem deterioration (Moss 2008, Withers *et al.* 2014, Wurtsbaugh *et al.* 2019). Runoff magnitude, dynamics and chemical composition are controlled by multiple factors that involve characteristics of rainfall, soils, topography, vegetation and land use (Hu & Li 2019). The mechanisms that define the fate of rainwater are relevant to understand runoff generation, infiltration, and erosion (review in Reid *et al.* 2018). In general, smoother surfaces promote runoff, explaining why in cultivated areas runoff is high (Simmonds *et al.* 2016, Zhao *et al.* 2018). Furthermore, the composition of runoff water is conditioned by soil use and vegetation cover, which alters nutrient concentration in the soil and their elemental relationships (Groppo *et al.* 2015). Nutrients can move from the soils to the water as they become dissolved in runoff water or moved in particulate form when bound to soil particles and suspended sediments (Wang *et al.* 2014).

Under this scenario, one of the most frequent strategies to reduce the external loads of nutrients to aquatic ecosystems is the maintenance or re-establishment of riparian zones (also referred through the text as buffer zones), which act as buffers, by trapping, eliminating, and/or transforming macro and micronutrients. Nutrients retention and removal occur through high particle deposition, uptake by plants, and soil microbial processes (Dosskey *et al.* 2010), and are influenced by climate, the width of the vegetated area, the slope of the banks, and the type of vegetation, among others (Zhang *et al.* 2010). It has been suggested that arboreal vegetation can be more effective in the removal of nitrogen (N) and phosphorus (P) than shrubs and grasslands (Zhang *et al.* 2010, Aguiar *et al.* 2015, Walton *et al.* 2020). In areas with riparian trees, where the soil is protected from the impact of raindrops, the infiltration rate is higher, and erosion is lower than in areas with herbaceous vegetation (Aguiar *et al.* 2015). A lower export of P from riparian zones with trees occurs regardless of rain intensity. For nitrogen, in contrast, there seems to be a significant role of the type of vegetation and soil microbial community in strong connection to rain intensity (Neilen *et al.* 2017). Water availability, and N and P content in the first layers of arboreal riparian soils can be significantly lower than those in soils with herbaceous cover, due to the high evapotranspiration rates of trees (Chen *et al.* 2003, Fortier *et al.* 2015) and higher microporosity due to larger roots. This mechanism reduces the mobility of nutrients from the soil to runoff water (Fortier *et al.* 2015).

Despite the widespread application of direct seeding practices has led to a reduction in soil erosion (Horowitz *et al.* 2010, Rusu 2014), it has also promoted the accumulation of P in the first 2 cm of soil profile, thus dramatically increasing the export of dissolved forms in runoff water (Sharpley *et al.* 2000, Tiessen *et al.* 2010). Thus, the top first layers strongly influence the composition of surface runoff water (Rowe *et al.* 2015, Smith *et al.* 2016, Baker *et al.* 2017), increasing the risk of water contamination due to the high accessibility of bioavailable forms for phytoplankton and macrophytes (Boström *et al.* 1988).

Since buffer zones represent narrow strips of different types of vegetation surrounded by crops, or by crops and aquatic ecosystems, they can behave in different ways under high nutrient loads. Nutrient retention can lead to an enrichment of buffer zone soils with labile forms of P, when compared to the adjacent agricultural fields, which suggests that under this circumstances soils in buffer zones can eventually behave as significant sources of both inorganic and organic dissolved P (Dodd & Sharpley 2016). Regardless of species composition and life forms, higher rates of P cycling and solubility occur in riparian vegetation than in adjacent cultivated areas (Stutter *et al.* 2009), likely because buffer areas soils (both arboreal and grasslands) have a higher content of organic matter and a high microbial activity (Roberts *et al.* 2013).

Extreme precipitation events increase drag and transport of nutrients from cultivated areas (Gao *et al.* 2014). Such impact is expected to increase in the context of climate change (IPCC 2014, Haylock *et al.* 2006, Westra *et al.* 2014, Ockenden *et al.* 2017). In particular, higher P and N loads are expected in water bodies, transported by surface and subsurface runoff, with seasonal variability depending on the local climate (Jeppesen *et al.* 2009, Jeppesen *et al.* 2011, Ockenden *et al.* 2016). This enhanced load of nutrients is one of the mechanisms by which further algal and cyanobacterial blooms are expected in the future (Paerl & Huisman 2008, González-Piana *et al.* 2017; Haakonsson *et al.* 2017, Meerhoff *et al.* 2022).

Long-term monitoring has shown that agricultural practices alter runoff and soil erosion processes, enhancing the loss of P when compared to riparian environments (Verheyen *et al.* 2015). Our study aimed to test the nutrient retention capacity of buffer zones, within the largest basin-level water-quality recovery plan Uruguay has implemented so far, to face the water quality crisis in the main drinking water source. Thus, we evaluated *in situ* surface and subsurface water of three different vegetated buffer zones (grasslands, shrubs, and forest), and the uphill adjacent crops for a whole year, to test their nutrient retention capacity under a wide range of natural precipitations. Based on international findings, we expected that the different types of riparian vegetation and their particular attributes would modify the physical-chemical characteristics of runoff water, since they vary in the magnitude and form of absorption and/or infiltration of sediments, nutrients and micronutrients from the area of drainage, while simultaneously providing organic matter. The forest was expected to retain a greater amount of nutrients from surface and subsurface runoff water than grassland cover (an intermediate effect is expected in the shrubland). On the other hand, higher intensity rainfall would expectedly reduce the "buffer" effect of the vegetation by increasing the transport of substances between the terrestrial and aquatic environment, with greater magnitude effects in grassland riparian zones (due to less protection from the direct impact of precipitation).

2. Methods

2.1. Site description and study design

The study was conducted in 2018-2019 in Paso Severino Reservoir, located in the Santa Lucia River Basin, Uruguay (34°12'12”S, 56°18'10”W). This is the biggest reservoir for drinking water in the country, which is operative since 1987 and supplies water to 60% of the Uruguayan population and numerous agro-industrial activities. Climate is humid subtropical with hot summers and without dry season (Köppen 1936, Peel *et al.* 2007), with an annual average temperature of 16.6°C and accumulated precipitation of 1257 mm in the study area (based on the historical 1991-2020 records from the Uruguayan Meteorological Institute). The seasonal distribution of rainfall is highly variable, as well as the rain intensity among precipitation events, resulting in the occurrence of periods of drought at any time throughout the year, despite evapotranspiration is much higher in summer. Dairy production and agriculture have historically been the predominant land use within the Paso Severino basin (Chalar *et al.* 2017, Arocena *et al.* 2018). In 2013, after a dramatic event of bad smell and taste caused by (non-toxic) cyanobacteria that jeopardized water access to Montevideo and the metropolitan area, several protection and rehabilitation measures were taken in the basin, through the “Action plan for the protection of environmental quality and available sources of drinking water in the Santa Lucía basin”. This is the largest basin-level water quality recovery plan implemented so far (DINAMA 2013). One of the actions was to fence a 100-m wide perimeter around the entire reservoir (as from 2016). Changing natural vegetation cover, crops, livestock, and the application of agrochemicals were prohibited in this aera, with the purposes of favoring the growth and regeneration of natural vegetation in the margins and of minimizing, in the long term, the input of nutrients and sediments transported by runoff and erosion into the reservoir.

For this study, three 100-m wide buffer areas inside the fenced area were selected based on the predominant vegetation cover: 1) grasses, 2) shrubs, and 3) native forest. All of them receiving runoff from adjacent land dominated by crops (artificial pastures of oat, sorghum, and clover) (Fig. 1). The sites (S) were defined as the three different pairs of crops + buffer. The grassland was dominated by *Cynodon* *dactylon*, an exotic and invasive grass species, while the larger plants were dominated by *Eryngium* sp. and some regeneration of the bush *Vachellia caven*, and many other small typical herbaceous plants of the region in lower abundances. In the shrubland, *V. caven*, *Senna corymbosa* and other subshrubs, mainly of the *Baccharis* genus, dominated in terms of abundance. Such a composition represents a vestige of the original, pre-agriculture, savanna ecosystem. The native forest was comprised of *Allophylus edulis*, *Schinus longifolius*, and *Scutia buxifolia*, among other native woody species, and also had a high density of large herbaceous plants, such as *Eryngium* sp. A high number of shoots of the invasive species *Fraxinus americana* and *Gleditsia triacanthos* were also found. Although vegetation was already established before the fencing, protective measures have promoted natural succession.

The three sites have typical Argiudoll soils according to the USDA classification. Selected farms experienced similar uses and management: annual crop rotations with multi-year pastures, without fertilizations during the sampling period. Topography at all three sites had an average slope of 3°.

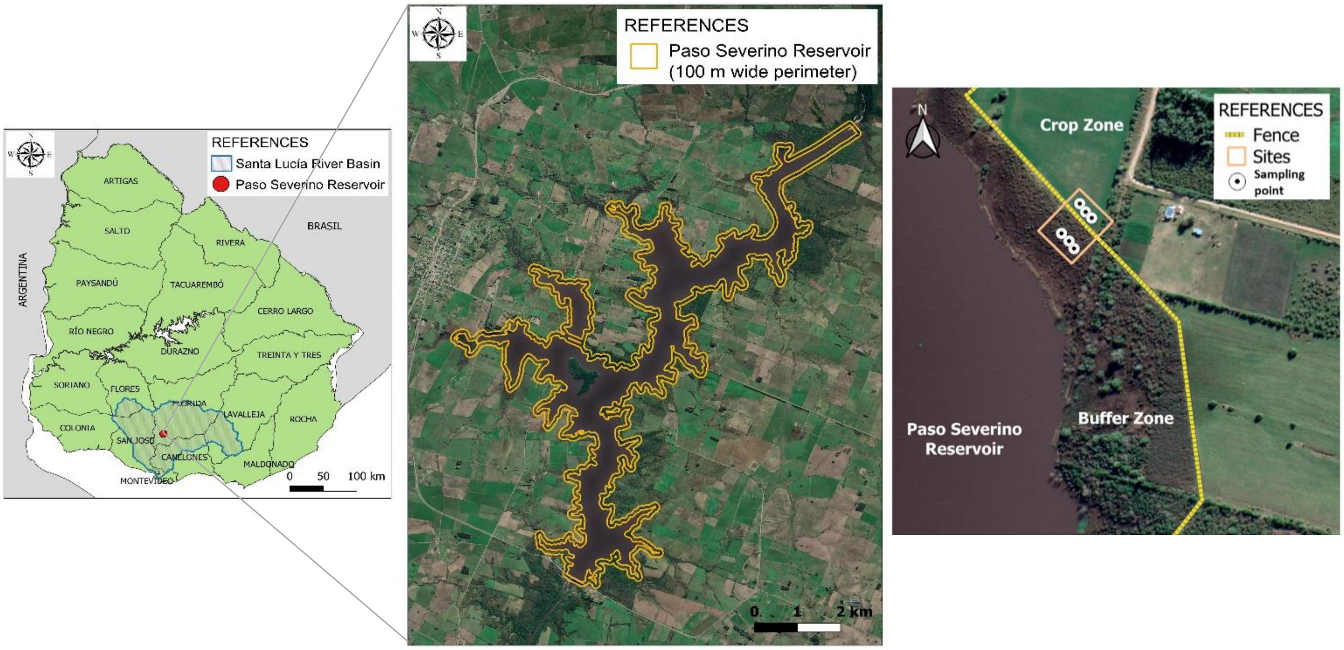


Figure 1. Map showing the study area location (34°12'12"S, 56°18'10"W), the fenced perimeter of the reservoir (in yellow) and the position of a surface and subsurface runoff sampling site (orange) and sample points (white) within crop and buffer zones.

2.2. Field sampling and laboratory analyses

Surface and subsurface runoff water samples were collected from August 2018 to July 2019, after storm events ranging from 19 to 101 mm. Meteorological data: air temperature, humidity, rainfall and wind intensity, were recorded at 15-minute intervals during the whole period (Rainwise Portlog) (Table A.1).

2.2.1. Soil physical-chemical analyses

Soil pits were dug to determine the structural and morphological characteristics of the different soils. To measure water extractable nutrients, composite samples of soils from 10 random points adjacent to the water sampling points were taken at three different depths (0-2.5 cm, 2.5-7.5 cm and 7.5-15.0 cm). We determined (at 0-5.0 cm and 5.0-10.0 cm) the concentration of organic carbon (Walkey & Black 1934), texture (% clay, sand, and silt; Bouyoucos 1927), apparent density (Burke *et al.* 1986), hydraulic conductivity in saturated flow (Klute & Dirksen 1986), and water content (gravimetric) of the soil. Subsamples of soil were oven dried at 105 °C for 3 days to determine moisture content.

2.2.2. Surface runoff

Surface runoff was artificially generated using a rainfall mini-simulator according to the methodology described in Kamphorst (1987) (Fig. A.1). The runoff simulation was conducted within 24-36 hours after the selected storm events. Therefore, the soil was at "field capacity" in all sampling events, i.e., when the drainage to deeper layers is very low and the water content of the soil is stabilized (Kirkham 2005). Although with this design the initial transport of compounds happening during a storm event was not captured, this methodology allowed us to explore the transport of nutrients some days after a long-lasting rainfall event typical of the region.

The simulator consisted of a sprinkler with 49 capillaries and a built-in pressure regulator necessary to produce a standard precipitation with uniform drops, which fall from a height of 0.5 m on a plot of 0.0625 m^2 (Iserloh 2013). Each simulation consisted of a high intensity 3-minutes rain (6 mm/min). Although it certainly does not reflect natural conditions in Uruguay, this high intensity is necessary to produce processes comparable to those that occur naturally during rainfall, such as particle entrainment. We performed four sampling campaigns (one per season), which consisted of three replicated simulations in each buffer zone and three simulations in the corresponding crop area. Shrubs and forest sites shared the same crop area, therefore only one set of three simulations was done (with a total of 15 simulations in each sampling event).

Simulation plots were randomly located within each vegetation of interest and aboveground vegetation was cut before the start to a height of 5 cm, to standardize the procedure. At the downslope end of each plot, a V-shaped drainage outlet was used to collect the surface runoff water in plastic bottles. In each simulation, the time until the beginning of runoff (i.e., first drop of water collected at the end of the plot), the volume of fallen precipitation and the volume of collected runoff water were recorded. The ratio between these last two variables determined the percentage of surface runoff. In addition, the percentage of bare soil of each plot was estimated through processing photographs taken at the site with the free software CobCal v2.0 (Ferrari *et al.* 2006).

The water collected was stored in clean and rinsed polyethylene bottles at 4°C for further analysis in the laboratory, where TP and PO_4^{3-} (method 4500-P), TN (method 4500-N.C), NO_{3-} (method 4500-B), NH_4 (method 4500-NH3) and suspended solids (SS), were analyzed according to standardized methodologies (APHA 1998). The nutrient loads were calculated by multiplying the concentration measured in the water collected in each simulation by the volume of runoff water in each plot (as in Borin *et al.* 2005).

2.2.3. Subsurface runoff

The subsurface water collectors consisted of a 110-mm diameter PVC bottom-sealed tube buried approximately 90 cm in the ground, with a 20-cm long section with small perforations (1 mm diameter) placed at the same height that the soil B horizon. At the top, a PVC elbow sealed with a mesh allowed the exchange of air and prevented the entry of rainwater and small animals. A separate shorter tube (200-mm diameter) buried in the first layers of the soil prevented the immediate surface runoff water from entering the tube, in order to ensure that only the water circulating in the soil's B horizon could enter the PVC pipe through the pores. Water was thus stored at the bottom of the tube until its collection (Fig. A.1) (methodology details in Calvo *et al.* 2020). An iron cage was placed in each dispositive as protection against potential cattle and passersby. The collectors were installed by triplicate in each crop area and the respective buffer zone (n total=18).

Sampling was performed eight times following significant rain events, between 24 and 36 hours after each rainfall. Temperature, % dissolved oxygen, pH, and conductivity of the stored water were measured *in situ* using a multi-parameter probe (MACRO 900, Palintest Ltd.). Also, we measured the water depth in each collector to calculate stored volume. The water retained in the collectors was then removed using a water pump connected to a 12V battery and stored in rinsed polyethylene bottles at 4 °C, until their processing in the laboratory as described above. Only dissolved nutrients, fraction most measured in subsurface water, were considered since clay particulates (and associated nutrients) move very slowly in the soil matrix through the porous system during water infiltration under unsaturated conditions and during water flows under saturated conditions (Hansen *et al.* 2002, Fredlund *et al.* 2010). Each collector was cleaned, and minimal maintenance tasks were performed after sampling. The nutrient load was calculated by multiplying the concentration of the collected water by the volume of water stored inside the collectors. The collection area of the devices was estimated at 0.1 m2, according to the type of soil and the hydraulic conductivity in saturated flow values associated with the different horizons.

2.3. Retention analysis

Comparing concentrations and loads in sites on the same slope is a broadly used approach (e.g., Balestrini *et al.* 2011, Aguiar *et al.* 2015, Saleh *et al.* 2018), and despite being a simplification, it can help understand nutrient dynamics and mechanisms acting at the basin scale. The percentage of nutrient retention by the buffer vegetations was estimated considering the loads measured in the crops uphill as 100% and, assuming this represents the nutrients load transported to the buffer zones. Meanwhile, the values measured in the vegetated buffers were assumed to be the remaining percentage of nutrients after filtering within the riparian buffer zone. The difference was assumed to be the mass of nutrients removed (negative difference) or enriched (positive difference) by the buffer zone.

2.4. Statistical analysis

We test for significant differences between sites (3 levels: crop + grassland, crop + shrubland, and crop + forest) and zones (2 levels: crop and buffer soils) in Bray-P, TN, organic carbon, organic matter, and conductivity using two-way ANOVAs.

We used two complementary approaches to analyze our runoff data. First, we evaluated the effects of site (3 levels: crop + grassland, crop + shrubland, and crop + forest) and zone (2 levels: crop and buffer) by testing for differences in mean surface (n event = 4) and subsurface (n event = 8) runoff water characteristics among treatments. For such testing we used two-way ANOVAs and Tukey's HSD function for pairwise comparisons. In surface runoff the tested variables were time to onset, volume collected, and concentrations and loads of TP, TN, PO_4, NO_3 and NH_4. For subsurface runoff the tested variables were PO_4 and NO_3 concentrations and loads. Shapiro-Wilk tests were conducted for normality check of residuals and Levene test for homoscedasticity check.

Secondly, we constructed generalized linear models (GLMs, *stats* package) and linear models (LM, *stats* package) to test for effects of potential explanatory variables related to climatic and soil characteristics on surface and subsurface runoff characteristics. For surface runoff the response variables were time to onset, volume of runoff water collected, and TP, TN, PO_4, NO_3 and NH_4 concentrations and loads. The explanatory variables were soil humidity, vegetation cover, runoff rate, organic matter content, Bray-P and N total in soil. For this analysis we pooled the three crops and the three buffers in one group to detect broad responses regardless of land cover. Before running GLM, we visually inspected the goodness of fit of the distribution function for each response variable in our data. The best fitting model was then chosen following Akaike's Information Criterion (AIC), where lower values are considered as better-fitted models (Sakamoto *et al.* 1986), after checking for test assumptions and residual distribution patterns, while variance explained (D^2) was calculated as deviance: 1- (residual deviance/null deviance). All models were subjected to a residual checking plots analysis to ensure that the GLM assumptions were met. Furthermore, a hierarchical partitioning was performed to determine the proportion of variance independently explained by each variable of all the variance explained using the package *hier.part* (Nally & Walsh 2004). To analyze the relation between surface runoff rate (L/min) and the concentration and load of TP, PO_4, TN, and NO_3, we used linear models (LM), choosing the best fit using AIC and deviance. Also, LM were used to analyze the relation between concentration and load of TP, PO_4, TN, and NO_3 and subsurface water volume, as well as with precipitation and ambient temperature. For the LMs, we pooled the three sites to enable detection of a potential overall difference between crops and buffers.

The significant differences of nutrient retention between the three buffer zones for both superficial and subsurface runoff were tested by one-way ANOVA (3 levels for site), followed by Tukey's multiple comparison tests. Shapiro-Wilk tests were conducted for normality check of residuals and Levene test for homoscedasticity. All statistical analyses were conducted using the open-source software R (R Studio Team, 2018).

3. Results

3.1. Soil characteristics

Crop soils had lower physical quality and chemical characteristics that differ from that of respective buffer zones. Concentration of available phosphorus varied significantly in relation to soil depth in both crop and buffer areas, with higher concentrations in the upper layers (Table A.2, Table A.3). Soil TN was significantly higher in forest than grassland. TN varied in relation to depth in both crop and buffer soils (Table A.2, Table A.3). The concentration of organic carbon was lower in the areas with crops, and overall higher in the forest site than in the grassland. Organic carbon was more abundant in the superficial layer of the soil for all covers (Table A.2, Table A.3). Higher soil apparent density and lower total porosity occurred in the crop only in forest, which promoted lower hydraulic conductivity. The grassland site had significant higher apparent density than shrubland and forest. The forest site presented an overall greater conductivity due to a high value in the buffer (significantly higher than in the crop). Percentage of clay did not show differences between sites, land covers or soil depth (Table A.2, Table A.3). The percentage of sand was greater in the crop than in the buffer zone within the grassland site, and was lower in both shrubland and forest. Silt showed the opposite pattern, with lower percentage in the crop than in the buffer zone within the grassland site, and higher in both shrubland and forest.

Crops from the three sites were similar, which allowed us to treat them as replicates for comparison with riparian buffer. When comparing buffer soils, forest showed the highest values of hydraulic conductivity in saturated flow, % of organic carbon, organic matter and nitrogen (Table A.2, Table A.3).

Soil humidity increased with accumulated precipitation previous to the runoff simulation (Fig. A.2), with precipitation explaining between 60 and 80% of variability in soil humidity across all study areas.

Vegetation cover in the crop plots was always significantly lower than in the buffers and varied among months, increasing towards the warmer months (Fig. A.2).

3.2. Surface runoff

Crops infiltrated less runoff water and registered higher loads of P being transported downhill than buffers. The forest cover was the only one that consistently showed reduced nutrient concentration and loads for both P and N. Differences between crop areas and buffer zones were found for most of the response variables measured, and in less magnitude also between sites. The starting time of runoff (onset) was significantly longer, and the volume of water collected was significantly lower in the three buffer zones (Fig. 2, Table 1). Onset was slower in the grassland site than in the shrubland site (Fig. 2, Table 1). In the forest zone, water fully infiltrated in the plot and there was no surface water flow in several simulations.

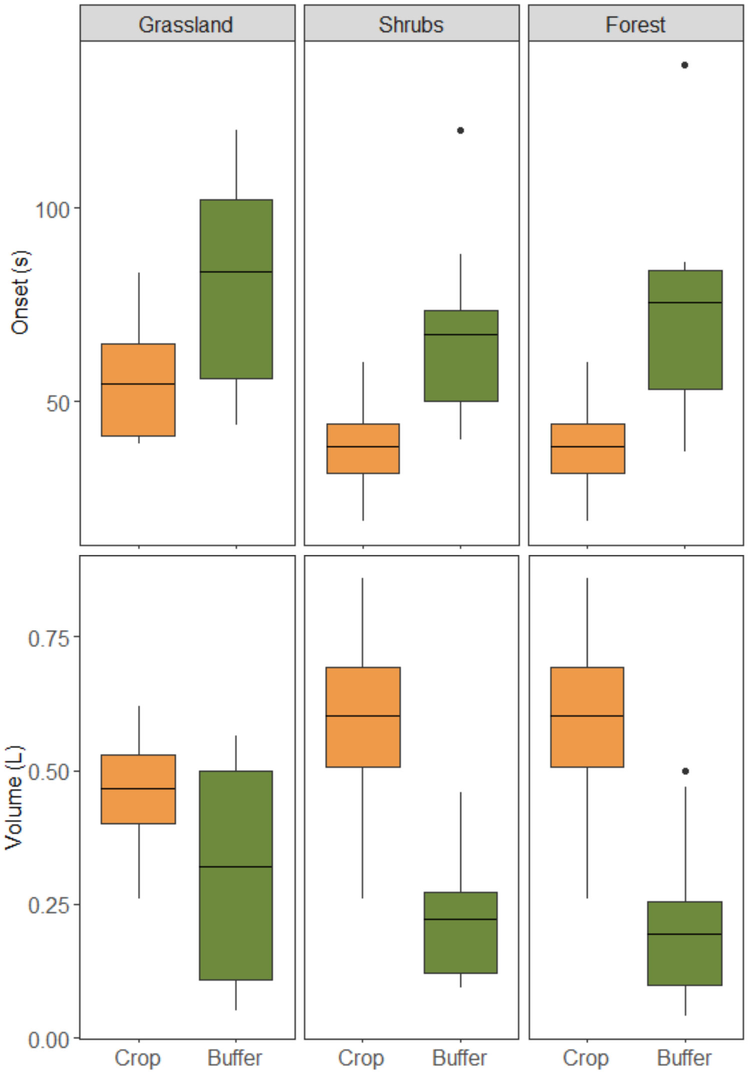
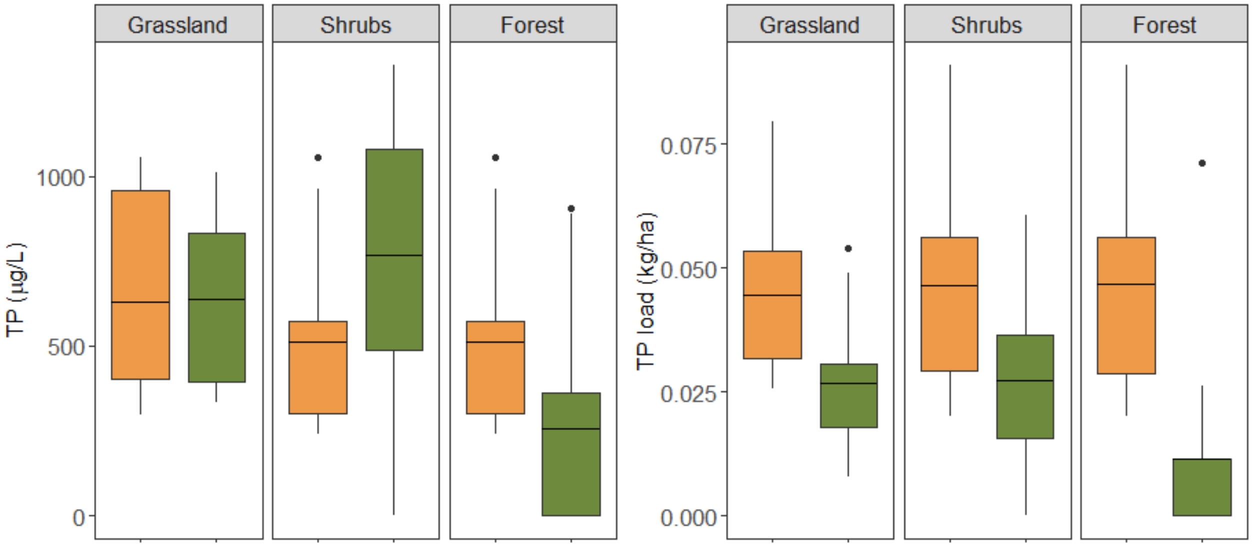
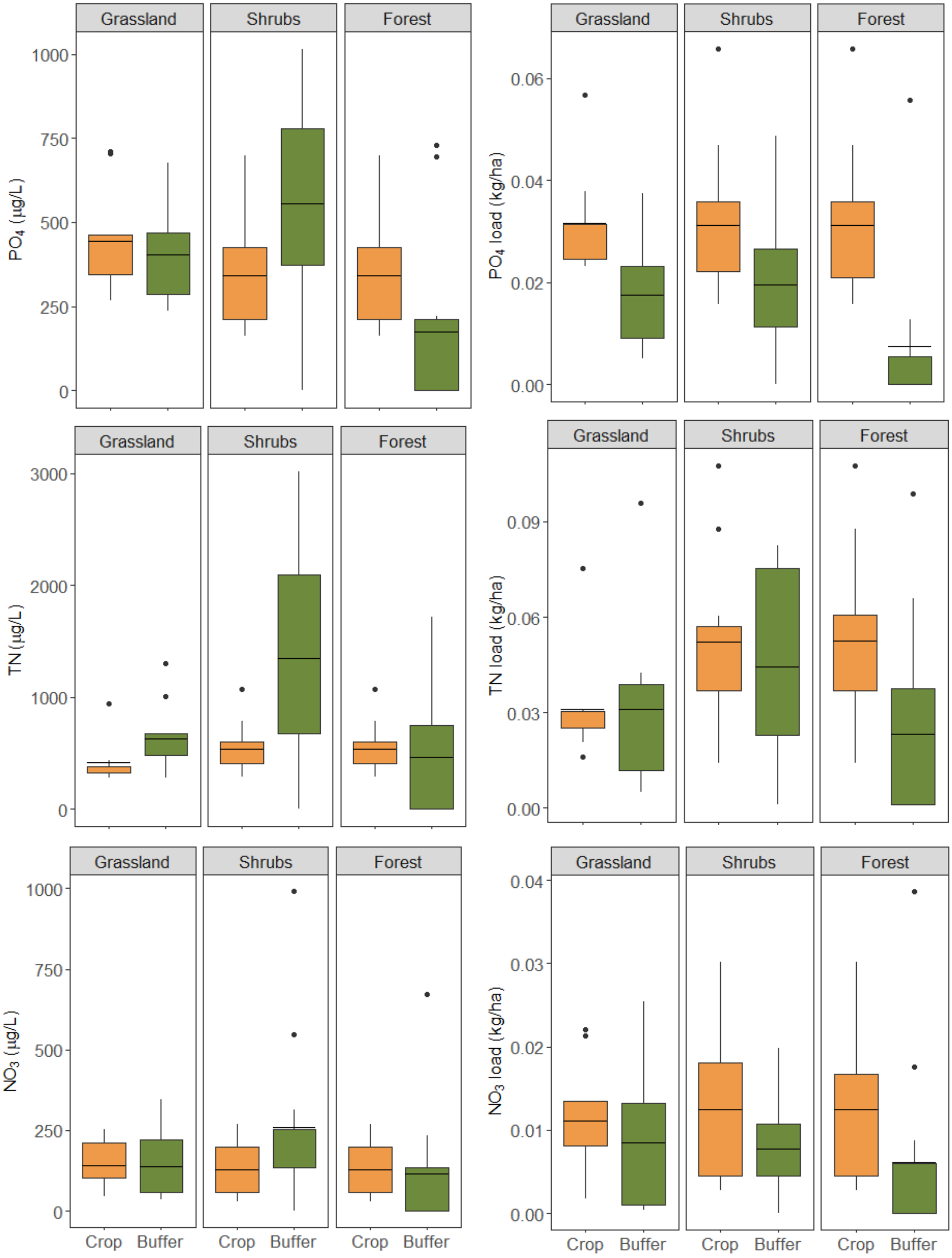


Figure 2. Time to onset (s) (upper panel) and runoff volume (L) (lower panel) of surface water in crops (orange) and buffers (green) of the three sites: grassland (left), shrubland (middle), and forest (right). Mean values, standard errors (boxes) and minimum and maximum values (whiskers) are shown.

In surface runoff TP and PO_4 concentration and load behaved differently. For concentration we registered overall higher mean values of both TP and PO_4 in the grassland than in the other two sites and an unexpectedly high concentrations in shrubs (Fig. 3, Table 1). The proportion of PO_4 in TP (µg/L) was in general high, with a global mean ca. 70%. Although it was not significantly different between zones, there was a trend of slightly higher values in crops. On the contrary, TP and PO_4 loads were significantly lower in the buffer zones than in the crop areas for all three vegetated buffers (Fig. 3, Table 1).

TN concentration and load tended to be higher in the buffer zones than in crops for grassland and shrubland sites but were lower in the buffer zone in the forest site (only significant for TN load, Fig. 3, Table 1). NO_3 concentration did not differ between crops and buffers for the three sites (Fig. 3, Table 1), while on the contrary, NO_3 load was significantly lower in the three vegetated buffer zones than in their respective crop areas (Fig. 3). NH_4 concentration was significantly higher in shrubland than in grassland and forest, and it tended to be higher in the buffer zone than in the crop in grassland and shrubland, and lower in forest (Fig. 3, Table 1). NH_4 load was significantly lower in the forest buffer zone than in its respective crop area. Suspended solids did not differ between sites or between zones (ANOVA, $p>0.05$).




Grassland
Shrubs
Forest
PO$_4$ (µg/L)
0
250
500
750
1000
PO$_4$ load (kg/ha)
0.00
0.02
0.04
0.06
TN (µg/L)
0
1000
2000
3000
TN load (kg/ha)
0.00
0.03
0.06
0.09
NO$_3$ (µg/L)
0
250
500
750
1000
NO$_3$ load (kg/ha)
0.00
0.01
0.02
0.03
0.04
Crop
Buffer

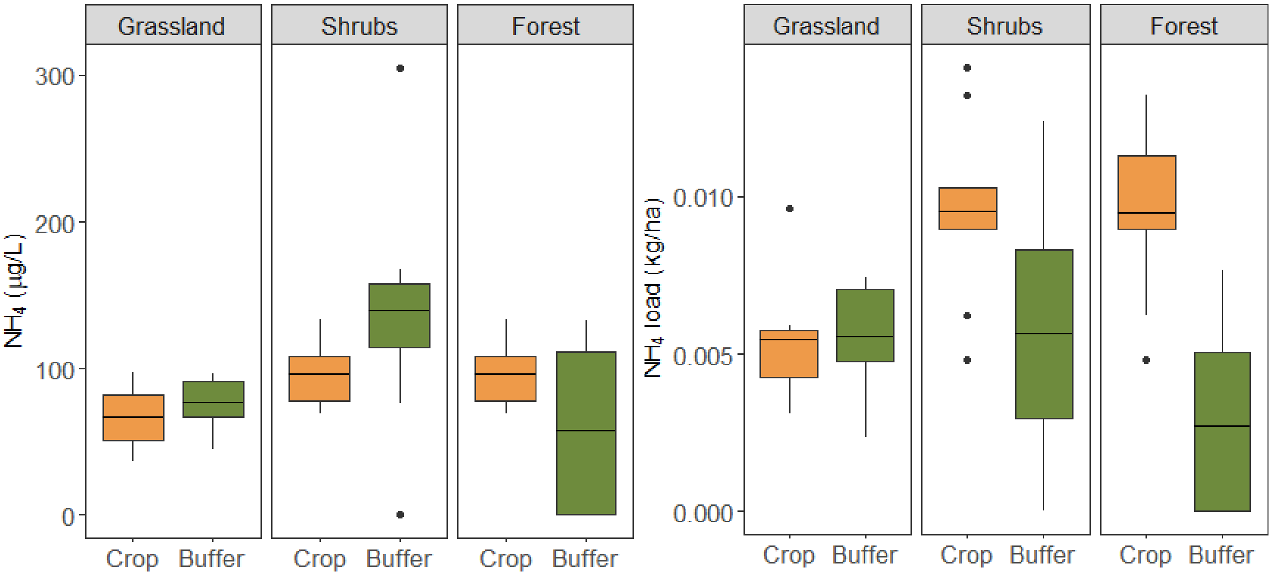


Figure 3. From top to bottom: TP, PO_4, TN, NO_3 and NH_4 concentration (µg/L) (left panels), and loads (kg/ha) (right panels), of surface runoff water in crops (orange) and buffers (green) of the three sites: grassland (left), shrubland (middle), and forest (right). Mean values, standard errors (boxes) and minimum and maximum values (whiskers) are shown.

Table 1. Results of two-way ANOVA testing main effects of factors: site (S) and zone (Z: buffer vs crops), and their interactions (S*Z), on runoff onset (s), runoff volume (L), TP concentration (µg/L), TP load (kg/ha), PO_4 concentration (µg/L), PO_4 load (kg/ha), TN concentration (µg/L), TN load (kg/ha), NO_3 concentration (µg/L), NO_3 load (kg/ha), NH_4 concentration (µg/L) and NH_4 load (kg/ha) in surface runoff, and PO_4 concentration (µg/L), PO_4 load (kg/ha), NO_3 concentration (µg/L) and NO_3 load (kg/ha). C=crop, B=buffer zone.

	Site			Zone				S*Z		
Surface	F	d.f.	*p*	F	d.f.	*p*		F	d.f.	*p*
Runoff onset	5.55	53	**	34.67	53	***	**C<B**			ns
Runoff volume			ns	72.76	60	***	**C>B**			ns
TP	5.03	60	**			ns		4.09	60	*
TP load			ns	24.93	60	***	**C>B**			ns
PO_4	5.51	60	**			ns		4.94	60	*
PO_4 load			ns	25.65	60	***	**C>B**			ns
TN	5.11	60	**			ns		5.06	60	**
TN load			ns	7.89	60	**	**C>B**			ns
NO_3			ns			ns				ns
NO_3 load			ns	9.56	60	**	**C>B**			ns
NH_4	4.41	60	*			ns		3.26	60	*
NH_4 load			ns	18.74		***	**C>B**	4.31	60	*

Subsurface										
PO_4	5.93	78	**	62.69	78	***	**C>B**			ns
PO_4 load			ns	40.66	78	***	**C>B**			ns
NO_3	2.97	78	.			ns		3.83	78	*
NO_3 load	3.4	78	*			ns				ns

Statistical results of ANOVA tests are shown, indicating F-values and degrees of freedom (d.f.) ns not significant $p>0.05$; *$p<0.05$; **$p<0.01$; ***$p<0.001$

When runoff characteristics (related to water dynamics and nutrients) from crops and buffers were pooled, we detected broad patterns showing that surface runoff was differentially explained by soil and climatic variables, with a prevailing role of vegetation cover (%) and soil humidity (Table 2). Soil organic carbon was relevant, explaining water infiltration. Onset was negatively related to runoff rate and positively with organic carbon in soil, while runoff volume was negatively related with both vegetation cover and organic carbon in soil. As expected, soil P largely explained both P concentration and load in runoff water (Table 2). Concentration and load of TP and PO_4 increased with Bray-P in soil. Higher vegetation cover (%) was associated with lower concentrations and lower loads of both TP and PO_4, while, as expected, more runoff rate increased loads. A reduction in soil humidity promoted higher values of TP concentration, as well as TP load. For nitrogen, an increase in vegetation cover and runoff rate promoted higher TN and NO_3 loads, while TN in soil increased only NO_3 loads (Table 2). None of the explanatory variables were significant for TN, NO_3 and NH_4 concentrations ($D^2< 0.05$, not shown). NH_4 concentration and load increased with TN in soil and soil humidity and decreased with runoff rate

No relationship between the TP and PO_4 loads with runoff rate was found in crops ($p<0.05$). In the buffer zones, however, load of both TP and PO_4 increased linearly with higher values of runoff rate (TP: $R^2=0.67$ and PO_4: $R^2=0.59$) (Fig. 4). TN, NO_3 and NH_4 loads increased significantly with runoff rate in both crops areas (TN: $R^2=0.36$, NO_3: $R^2=0.28$, NH_4: $R^2=0.24$) and buffer zones (TN: $R^2=0.40$, NO_3: $R^2=0.44$ and NH_4: $R^2=0.64$, respectively) (Fig. 4).

Table 2. Main effects of explanatory variables on surface runoff response (GLM models). Explanatory variables: vegetation cover (%), soil humidity, runoff rate, organic matter content, Bray-P in soil, TN in soil, and their interactions. Response variables: time to onset (s), runoff volume (L), and TP, and PO_4 concentration (µg/L) and TP, PO_4, TN, NO_3 and NH_4 load (kg/ha). *Represents a significant interaction between factors. The percentage of deviance explained by the model is shown (D^2).

Model	Predictors of model	Estimate	SE	t	*p*	I	D^2
Onset ~ Runoff rate + Organic carbon	Runoff rate	-3.79	0.63	-5.99	***	71.21	0.54
	Organic carbon	0.14	0.05	2.76	**	28.79	
Volume ~ Organic carbon + Vegetation cover	Organic carbon	-0.35	0.11	-3.26	**	60.77	0.36
	Vegetation cover	-0.01	0.004	-2.54	*	39.23	
TP ~ Bray-P * Runoff rate + Vegetation cover * Soil humidity	Bray-P	0.07	0.02	3.15	**	53.57	0.33
	Runoff rate	7.83	3.7	2.12	*	2.38	
	Vegetation cover	-0.02	0.01	-1.24		25.87	
	Soil humidity	-0.04	0.01	-2.28	*	18.17	
TP load ~ Runoff rate + Bray-P + Vegetation cover * Soil humidity	Runoff rate	143.84	17.25	8.36	***	83.8	0.57
	Bray-P	5.13	1.96	2.62	*	7.39	
	Vegetation cover	-2.48	2.23	-1.11		5.36	
	Soil humidity	-6.7	2.3	-2.91	**	3.46	
PO_4 ~ Bray-P + Vegetation cover	Bray-P	0.04	0.01	3.99	***	71.51	0.24
	Vegetation cover	-0.01	0	2.97	**	28.48	
PO_4 load ~ Bray-P + Runoff rate * Vegetation cover	Bray-P	4.53	1.25	3.63	***	11.81	0.60
	Runoff rate	152.61	73.82	2.09	*	81.58	
	Vegetation cover	-2.06	1.44	-1.43		6.6	
TN load ~ Runoff rate * Vegetation cover + Soil humidity * Vegetation cover	Runoff rate	0.77	0.26	2.9	**	79.72	0.45
	Vegetation cover	0.004	0.006	0.61		4.75	
	Soil humidity	-0.008	0.005	-1.57		15.53	
NO_3 load ~ Runoff rate + TN soil + Vegetation cover	Runoff rate	0.09	0.15	5.93	***	83.2	0.41
	N tot	0.03	0.01	2.16	*	9.52	
	Vegetation cover	0.001	0	2.06	*	7.28	
NH_4 load ~ TN soil + Runoff rate + Soil humidity	N tot	6.21	0.04	2.41	*	30.04	0.66
	Runoff rate	0.03	0.05	5.57	***	62.95	
	Soil humidity	0.001	0	2.18	*	7.01	

Ns not significant $p>0.05$; $*p<0.05$; $**p<0.01$; $***p<0.001$

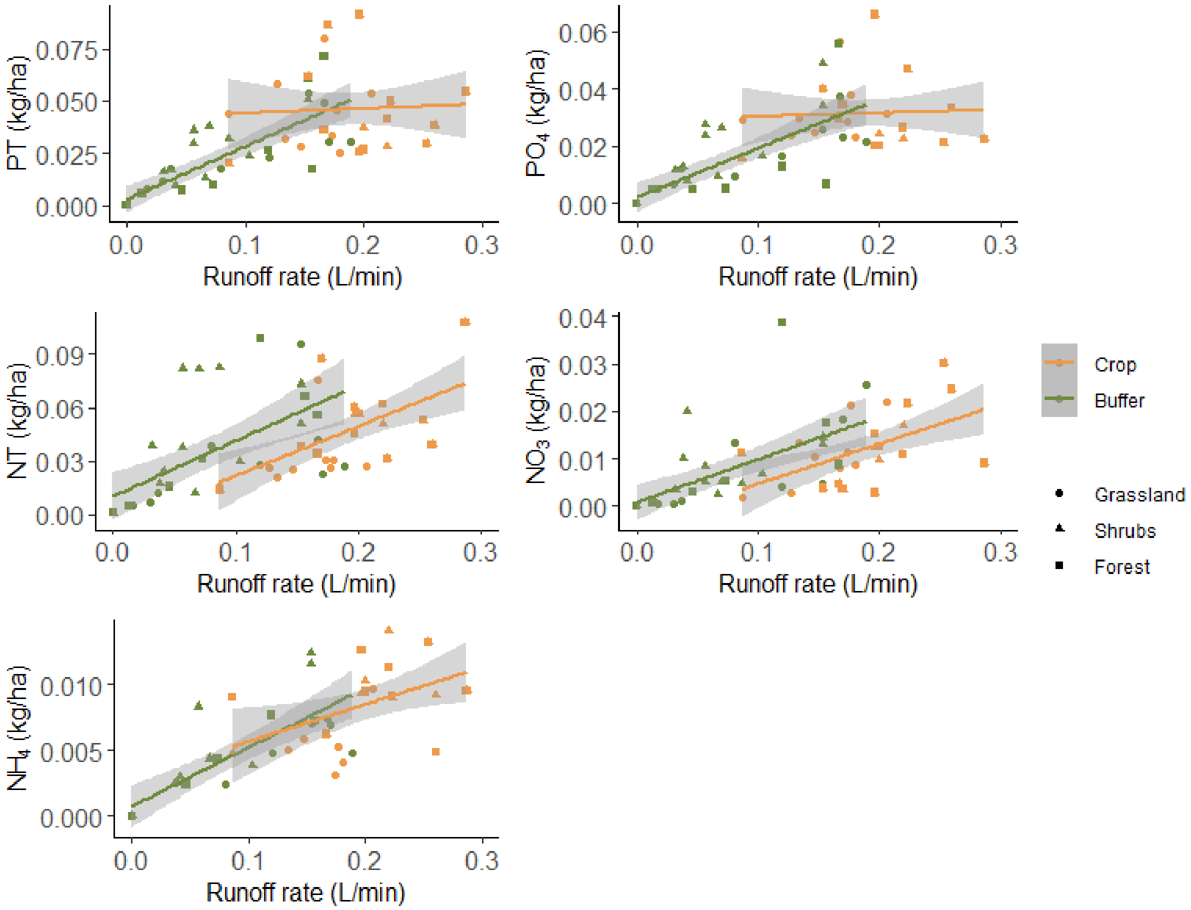


Figure 4. Graphical output of the linear regressions between load (kg/ha) of total phosphorus (TP), phosphate (PO_4), total nitrogen (TN), nitrate (NO_3) and ammonium (NH_4) in surface runoff water and runoff rate (L/min). Orange dots and lines represent crops and green ones the buffers. Data from the three sites were pooled.

3.3. Subsurface runoff

The PO_4 concentration and load in subsurface runoff were significantly lower in the three buffer zones than in the crop areas, and concentration overall lower in the forest site (Fig. 5, Table 1). In contrast, subsurface NO_3 in buffer zones revealed a significant interaction between site and zone. Grassland buffer had lower NO_3 concentration (Tukey's test, $p = 0.05$) than the adjacent crop area, load showed a similar but nonsignificant trend. In the shrubland and forest zones, the trend was the opposite, with higher but not significant values of NO_3 concentration and load measured in the buffers (Fig. 5, Table 1). NH_4 concentration and loads is subsurface runoff water were negligible and are not shown (in average less than

18% of NO_3 measured). Neither the concentration nor the load was significantly different between sites or zones (two-way ANOVA, $p>0.05$).

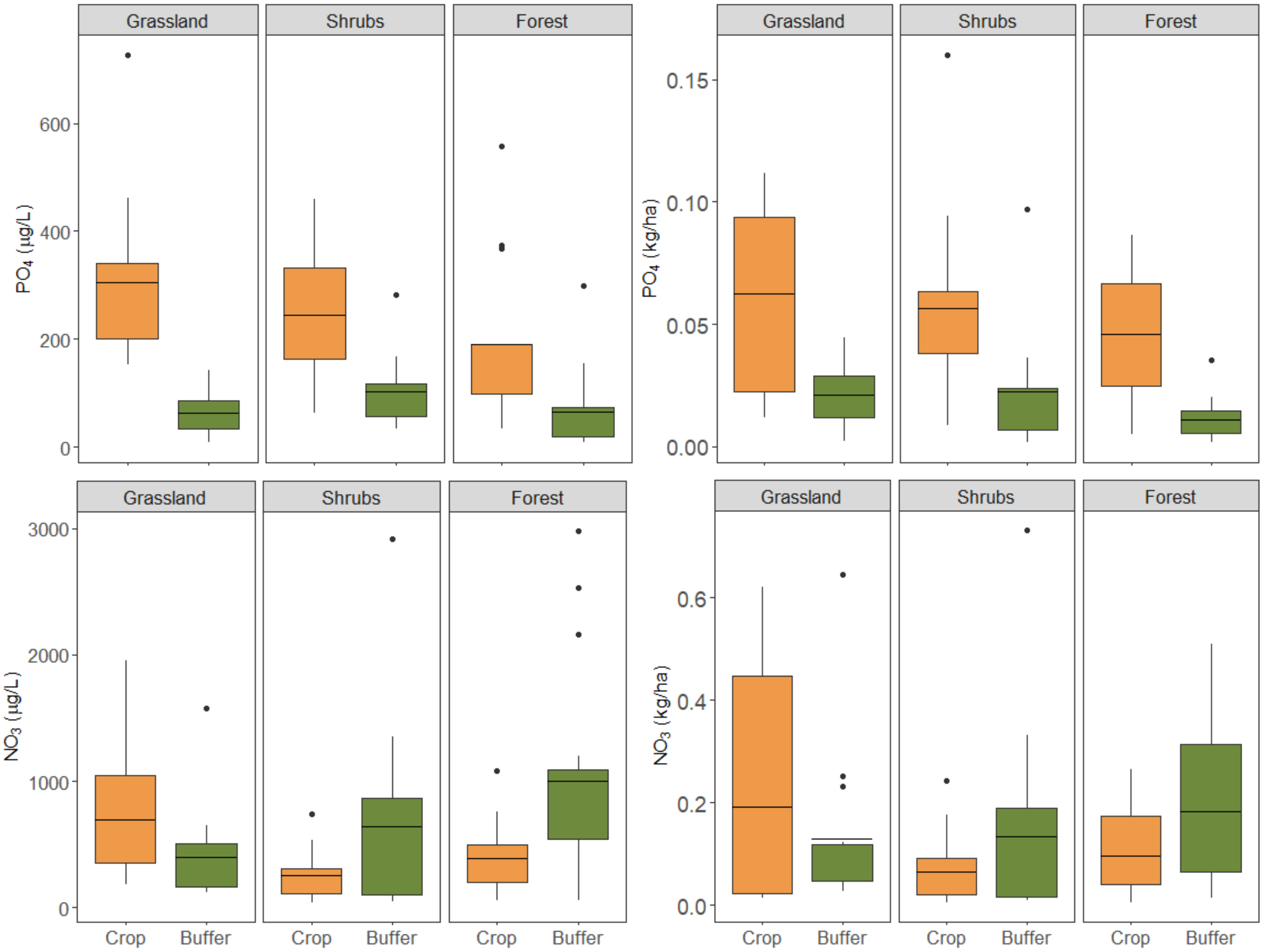


Figure 5. Top panels: Subsurface phosphate (PO_4) concentration (µg/L) and load (kg/ha), bottom panels: NO_3 concentration (µg/L) and load (kg/ha) in crops (orange) and buffers (green) of the three sites: grassland (left), shrubland (middle), and forest (right). Mean values, standard errors (boxes) and minimum and maximum values (whiskers) are shown.

We further explored nutrient dynamics in relation to gradients of precipitation and ambient temperature. In subsurface runoff water moving through crops, PO_4 concentration increased with ambient temperature ($R^2=23$, $p<0.001$), and PO_4 load increased with precipitation ($R^2=21$, $p<0.001$). In buffer areas there was no clear relationship with these environment variables (Fig. 6). PO_4 load increased with subsurface water volume for both crop and forest ($R^2=39$, $p<0.001$ and $R^2=14$, $p<0.05$, respectively) (Fig. A.3). NO_3 concentration tended to decrease with temperature (Fig. 6), in both crop and buffer areas, but relationships were statistically non-significant. NO_3 load tended to decrease with temperature in both crop (R^2=14, $p<0.05$) and buffer (R^2=17, $p<0.05$) areas. In contrast, no trends were found with precipitation. NO_3 load increased with subsurface water volume for both crop and forest (R^2=41, $p<0.001$ and R^2=13, $p<0.05$, respectively) (Fig. A.3).

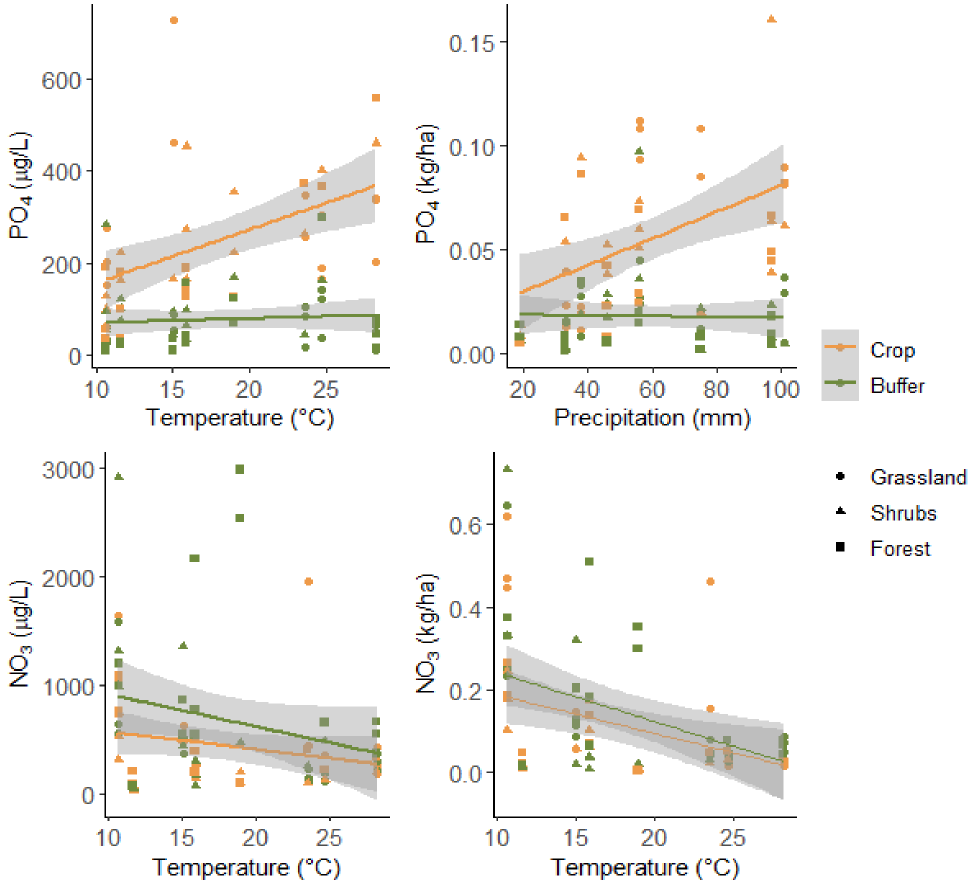


Figure 6. Top-Left: Relationship between PO_4 concentration (µg/L) and temperature (°C) in crops and buffers. Top-Right: Relationship between PO_4 load (kg/ha) and precipitation (mm) in crops (orange) and buffers (green). Bottom: Relationship between NO_3 concentration (µg/L) (Left) and NO_3 load (kg/ha) (Right) with temperature (°C) in crops (light grey) and buffers (dark grey).

3.4. Retention capacities

Riparian buffer zones effectively retained the load of PO_4 in both surface and sub-subsurface runoff but were less effective in reducing its concentration. In surface runoff, the greatest reduction in PO_4 concentration among buffer zones was in forest (~50%). PO_4 change was close to zero in the grassland, and increased in the shrubland (one-way ANOVA, $p<0.05$) (Fig. 7A). PO_4 load retention was greatest in the forest zone (~80%), however, no statically significant difference was observed among the three areas. Subsurface water revealed between-area differences in PO_4 concentration and load retention by the three buffer zones, with 50% as the overall mean retention for the different riparian vegetation (Fig. 7B). Total nutrient retention in surface water was greatest in the forest area. TP retention was -8, -65 and 53% in grassland, shrubland and forest, respectively (ANOVA, $p<0.05$), TP load was 25, 34 and 78% in grassland, shrubland and forest, respectively (ANOVA, $p>0.05$, positive % indicates retention by buffers, and negative % indicates enrichment).

The effect of buffer zones was more variable for NO_3, which acted as either a nutrient sink or source depending on the type of vegetation and runoff layer (surface vs. sub-subsurface). In surface runoff, we found no significant differences in NO_3 concentration among buffer zones, despite in the grassland zone the concentration was reduced (~25%) and shrubs and forest buffers appear to release NO_3. However, the NO_3 load was clearly retained in the three buffer zones, being maximal in grassland with ~50% (ANOVA $p>0.05$) (Fig. 7A). For total nitrogen concentration the retention was -68, -146 and 21%, (ANOVA, $p<0.05$) and for load -11, 18 and 62% (ANOVA, $p>0.05$), for grassland, shrubland, and forest, respectively. Meanwhile, in subsurface runoff, NO_3 concentration was only reduced in grasslands (ANOVA, $p<0.05$; Tukey, Grassland>Shrubs*), while the concentration in shrubs and forest, and load in all three buffer zones increased, suggesting a release of NO_3, although no significant differences were observed among buffer zones (Fig. 7B). For NH_4, the significant retentions registered were for load in surface runoff water in shrubland and forest (41 and 72%, respectively; ANOVA, $p<0.05$).

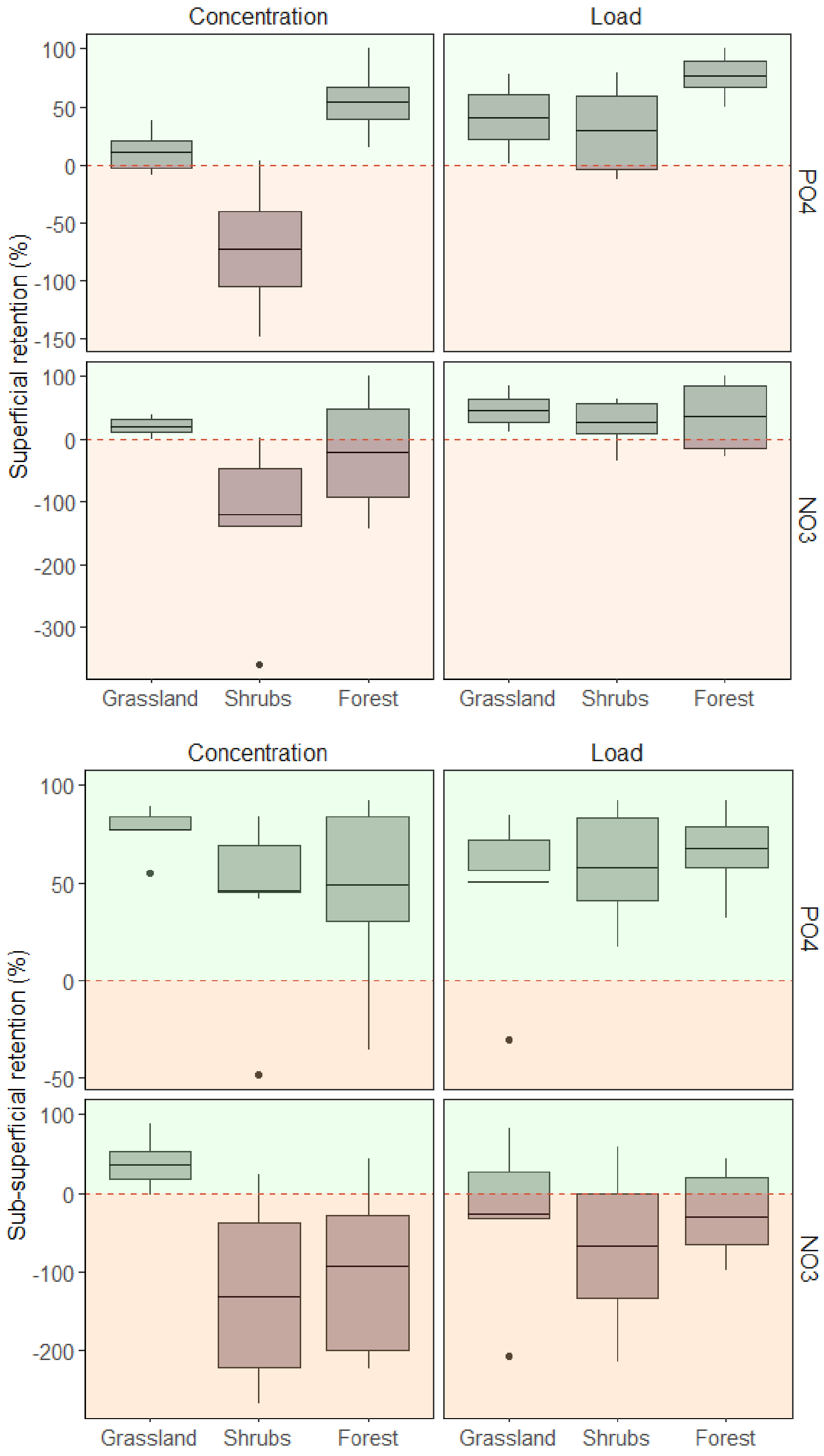


Figure 7. Surface (top) and Subsurface (bottom) potential retention of phosphate (PO_4) and nitrate (NO_3) concentration (µg/L) and load (kg/ha) in runoff water for the three sites studied: grassland, shrubland, and forest. Mean values and standard errors are shown. The green area shows the nutrients removed (positive difference) and the red area the nutrient enriched (negative difference) by the buffer zones.

4. Discussion

We found strong evidence of nutrient retention by buffer zones and consequent improvement of agricultural runoff water quality. All three types of vegetation cover (i.e., grassland, shrubland, and forest) decreased the amount of water reaching the reservoir and thus reduced dissolved and particulate nutrient loads. Among the three, forest cover enhanced phosphorus retention, while grassland was the strongest regulator of nitrogen dynamics.

Physical properties of soils in adjacent crop fields had lower quality than soils in riparian buffer areas. Soil with higher bulk density caused a faster start of surface runoff and a greater amount of water moving across the surface than in buffer zones. Soil physico-chemical characteristics, strongly affected by land use, influence infiltration and runoff (Boizard *et al.* 2013, Alaoui *et al.* 2018). During our one-year study, vegetation cover in cultivated lands was low in certain periods, creating conditions that generally reduce time required to onset runoff (Zhang *et al.* 2019). Similar to our findings, Aguiar *et al.* (2015) found high infiltration in riparian buffer areas, with maximum values in wooded areas, followed by shrubs and finally herbaceous vegetation.

Although simulated runoff has been shown to underestimate P concentration due to the short contact time between soil and water, it nonetheless can be a useful approach to understand the mobilization of compounds in soils (Dougherty *et al.* 2004, Dunkerley 2008). Even with potential underestimation, P concentration values were high in surface runoff from crops. Recent studies have found high TP concentrations in the reservoir water, with an average of ~300 μg/L during the period 2004-2016 (Aubriot *et al.* 2017), with a strong correlation between water TP and agriculture- land cover, and an inverse correlation between TP and forest- land cover (Gorgoglione *et al.* 2020), although both native forest and forest plantations were mixed in the analysis. In our study, surface PO_4 represented up to 75% of the TP in crops, suggesting that P was transported mainly as dissolved, directly bioavailable, form (Goyenola *et al.* 2015). In the subsurface runoff water, the concentration of PO_4 was lower than in the surface runoff, but the loads transported were substantial as well. Despite its low solubility within deep water-saturated soil, oxygen decreases and reduction processes may occur that enhance the release of soluble P into soil-water (Zhang & Furman 2020). The concentration of TP in top soil layers and groundwater are correlated across various land uses, which suggest a potential for vertical leaching of phosphorus to groundwater (Liao *et al.* 2019).

According to our results, forested riparian vegetation would result in less TP and PO_4 being transported from crops to water bodies via surface and subsurface water movement. Lower PO_4 concentration in surface water within the riparian buffer were only observed in our forested zone. Reduced loads and retention of PO_4 was observed in all three buffer zone types (with maximum in the forest). In general, greater substrate rugosity in riparian zones reduces runoff velocity, promoting increased infiltration and therefore reducing transfer of pollutants to aquatic ecosystems (Markin *et al.* 2007, Duchemin & Hogue 2009). In our study, P dynamics were strongly related to vegetation cover and soil humidity, both conditioned by the prevailing type of vegetation and likely their different effects on soil P sorption (Cao *et al.* (2019). In areas dominated by woody vegetation accumulated organic matter promotes phosphorus retention (Aguiar *et al.* 2015).

In contrast to P, subsurface water had higher nitrate concentrations than surface runoff in both crops and buffers, as expected (Bechman 2014). Nitrate is highly soluble and mobile, and it has been found to be mainly transported by subsurface flow (Simmons *et al.* 1992). We registered a reduction in nitrate concentration only in the grassland, both in surface and subsurface runoff. Nitrate load was reduced in the three buffer zones in the surface runoff, and only reduced in the grassland in the subsurface layer. Moreover, in shrubland and forest, we registered particularly higher subsurface concentration and load values in the buffer zones than in the respective crops. In various meta-analyses, the effectiveness of nitrogen removal has been shown to vary widely, with more consistent reduction values in water moving sub-superficially (Mayer *et al.* 2007, Valkama *et al.* 2019). Shallow subsurface flow through riparian zones can highly affect nitrate concentrations, as it moves through plant roots and microbial populations that promote and maximize immobilization and denitrification (Dosskey 2001, Lasagna *et al.* 2016). Williams *et al.* (2014) found lower NO_3 concentration near the surface (20 cm deep) than in a deeper layer of riparian soil (60 cm) and assumed that removal may be more efficient in near-surface riparian soils due to their higher content of organic matter. Nitrate retention has been documented in deeper layers of the soil, even for narrow herbaceous and woody zones through vegetation uptake and denitrification; although the latter seems less strong than in the first layers (Balestrini *et al.* 2011). The low nutrient retention and even exportation from the shrubland buffer zone may be related to the recent agricultural history of the area, whit crops up to the water margin until the fencing of the exclusion area. Vegetation and soil recovery may take several years, and thus the increase of the removal capacity, as vegetation succession continues and natural processes recover.

Overall, we found less phosphorus exported to watercourses from forested riparian zones than from zones with herbaceous cover, and the opposite pattern for nitrogen, mainly nitrate, as was broadly also found by Neilen *et al.* (2017). In our study area, a mix of herbaceous and woody species would enhance overall nutrient retention, already suggested for other regions (Cao *et al.* 2019), although studies have report different effectiveness and it does not seem possible to generalize (Prosser *et al.* 2020). Deep-rooted woody vegetation buffer is efficient in trapping soluble nutrients, due to the high infiltration capacity, while grass buffer seems effective in trapping coarse sediments and sediment-bound nutrients (Lee *et al.* 2000). Despite the important role of width (Zhang *et al.* 2010, Aguiar *et al.* 2015), more recently also interpreted as the ratio of source area to buffer area (Prosser *et al.* 2020), vegetation type has been found in some cases to have the highest influence on mass removal of phosphorus and nitrogen (Mankin *et al.* 2007). In our study site, the width of the riparian vegetation was almost invariable which allowed us to compare the effects of vegetation type per se. The potential changes in effects as a result of different strip widths should eventually be assessed. Besides the type of vegetation and width, various other factors can influence the efficiency of buffers in reducing the movement of nutrients (recent review in Prosser *et al.* 2020) and should be considered when planning and managing these areas (Mander *et al.* 2017).

We found some evidence on how the increase in precipitation and, to a lesser extent, the increase in temperature, could impact nutrient dynamics. For agricultural lands in many locations worldwide, increased precipitation is expected to increase the flux of nutrients to freshwaters because of increased runoff (Bender *et al.* 2018, Carpenter *et al.* 2018), through many interactions with surface cover, litter, and soil exposure (Puntenney-Desmond *et al.* 2020). Increasing rainfall intensity augment raindrop energy and surface runoff velocity (Wang *et al.* 2014), and usually for bare agricultural soils, there is a clear positive correlation between rainfall amount and intensity and runoff rate (Wang *et al.* 2014), and also with TP load and concentration (Ding *et al.* 2017). Here, the values for TP and PO_4 loads exported in surface runoff from crops were not related to the magnitude of runoff rate, probably because during the whole period, although the change was not substantial, the crops grew and on average the area increased its vegetation coverage from 70% to more than 90%. Meanwhile, TN, NO_3 and NH_4 loads in crops were positively related with runoff rate. Runoff rate, together with soil erodibility and agricultural management practices largely control superficial nitrogen losses (Zhang *et al.* 2016).

In the buffer zones, regardless of the predominant type of vegetation, the surface transport of both P and N increased with increasing runoff rate. Availability of nutrients in runoff has been found to be more influenced by runoff rate rather than by soil nutrient concentration or other soil properties (Wang *et al.* 2014, Zhang *et al.* 2019). In areas where more frequent extreme rainfall events are predicted this could be particularly relevant (Ockenden *et al.* 2017) and likely affect nutrient retention by riparian vegetation. In buffer areas, soil P cycling is enhanced and soil P solubility increased, leading to a high risk of dissolved phosphorus leaching into water bodies during rainfall events (Stutter *et al.* 2009, Stutter & Richards 2012), even bigger if leaf litter is accumulated in the soil (Cabrera-Lamanna 2019, Franklin *et al.* 2020). The removal efficiency of buffer zones, regardless of the type of vegetation cover, is negatively correlated with the amount and intensity of precipitation (Bu *et al.* 2016), which is also relevant under future climatic scenarios. Zhang *et al.* (2019) found that particulate phosphorus load is predominantly transported through the soil at the early stage of storm events, while dissolved P concentrations tended to be constant and, hence, make a more important relative contribution to TP towards the end of each precipitation event.

In subsurface water, while no trend was found in the buffer zones, PO_4 load increased with precipitation in the crop area. Buffer phosphate seems to be less subject to climatic variations than the cultivated zones, although the scarcity of data makes it difficult to draw robust conclusions. In this sense, a previous study with P leaching loss from riparian zones found that the lower export of phosphorus from riparian areas with trees (compared to areas without riparian vegetation) occurs regardless of rainfall intensity (Neilen *et al.* 2017).

In our study, air temperature was associated with water PO_4 concentration in crops and for both NO_3 concentration and load in buffers and crop zones. Removal efficiency of nitrate in buffer zones seems higher in summer than in winter likely due to the enhanced activity of denitrifying bacteria (Zak *et al.* 2018). Although recent models have predicted that, as long as water demand of plants is fulfilled, warming will increase future phosphorus uptake by crops and hence lead to less nutrients available be transported to water bodies, at the same time increased precipitation will still increase total dissolved phosphorus loss

(Kalsic *et al.* 2019, Wang *et al.* 2021), so that the magnitude and direction of the overall effect is difficult to predict.

The conservation and management of vegetated buffer zones, combined with a widespread and efficient application of best management practices for agriculture and animal production to reduce nutrients export, is essential to reduce the input of particulate and dissolved nutrients to water bodies and mitigate their impact (Sharpley *et al.* 2006, Sharpley *et al.* 2015, Arocena *et al.* 2018). Some management practices frequently used that involve tillage or manure are more effective in reducing erosion than runoff (Martinez-Mena *et al.* 2020). This is useful in many situations when P is mostly lost in its particulate form during rainfall events (Bender *et al.* 2018), but in our study site the predominant form was the dissolved one. Dissolved nutrient losses are the hardest to be recognized by farmers and are not targeted by the widespread management practices aiming at reducing erosion and particulate phosphorus loads (Dodd & Sharpley 2016). Zhang *et al.* (2019) found that particulate P was the dominant fraction (> 80%) of total P loss when the soil is completely bare, but dissolved P was dominant in the presence of an herbaceous cover (because of reduced erosion). Only with actions to mitigate erosion in a plantation, there will be still a lot of P loss.

In our study case, the restoration of the buffer area was only implemented along the reservoir shoreline, but extending the measure to tributaries and especially to headwater streams, could be equally or even more needed to maximize the retention, as these areas are more relevant for nutrient removal at the basin scale (Correll 2005). In addition, conservation practices to maximize riparian buffers effectiveness should be aimed (Yasarer *et al.* 2017, Wagena & Easton 2018, Martinez-Mena *et al.* 2019), for example, the inclusion of site-specific information and accounting for preferential flow processes (Dąbrowska *et al.* 2018, Hester & Fox 2020), and the removal of vegetal biomass. Here, cattle were completely excluded from the buffer area, but the entry of livestock during short periods of time could be a management option to reduce biomass accumulation if animal density and time frame are considered (Cole *et al.* 2020). Furthermore, a periodical harvest of vegetal material from buffer zones does not decrease buffer effectiveness in nutrient and sediment removal, and can even increase nutrient retention rate by P uptake (Hille *et al.* 2018, Jiang *et al.* 2018). Also, as discussed before, if plant community composition is strategically chosen when buffers are implemented and maintained, a greater efficacy may be achieved with a narrower strip, thus improving the balance between aquatic ecosystem protection and productive purposes (Prosser *et al.* 2020).

5. Conclusions

Large amounts of phosphorus are transported from crop fields to the Paso Severino Reservoir, mostly in dissolved forms through runoff water. We found that riparian buffer zones retain significant amounts of nutrients, and this was the case for all three classes of vegetation cover. Study results suggest that that buffer areas comprised of mixed herbaceous and woody vegetation would achieve greatest retention of phosphorus and nitrogen.

Our results show that the large-scale management measures applied will most likely decrease the direct input of nutrients into the reservoir, however, other factors must be considered. Current models for climate change project increased precipitation in the study region (Haylock *et al.* 2006, Reyer *et al.* 2015, Brêda *et al.* 2020), which may reduce buffering capacity in the riparian zone. In accordance with our results, previous studies have concluded that changes in precipitation and land uses that increase nutrient loading are likely to exacerbate eutrophication (Ockenden *et al.* 2016, Sinha *et al.* 2017). Thus, effective management of water quality requires consideration of agricultural practices, but also additional action. For example, buffer strips between paddocks may reduce the transport of nutrients to buffer zones (Sahu & Gu 2009), making them more efficient in the long term. To reduce reservoir eutrophication will require management at the whole-catchment level within the context of climate change (Nobre *et al.* 2020). Our findings emphasize that management also requires a focus on the dominant form of nutrients in runoff (Dodd & Sharpley 2016). Future research should aim at understanding the role that increasing runoff interacting with increasing temperature and future agronomic practices will exert on loads exported from the basin to water bodies.

Acknowledgments

We acknowledge the funding of DINAMA (former MVOTMA, current MA) for funding the project and the collaboration. Clementina Calvo acknowledges the Agencia Nacional de Investigación e Innovación (ANII, POS_NAC_2016_1_130095) and CAP-Udelar for funding her PhD. MM acknowledges the support of ANII, PEDECIBA, and CSIC grant I+D 511. We especially thank Mr. Gerald Atrio for his valuable help in the field, and we thank Kirk Winemiller for helpful comments on an earlier version of the manuscript.

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<u>Appendix</u>

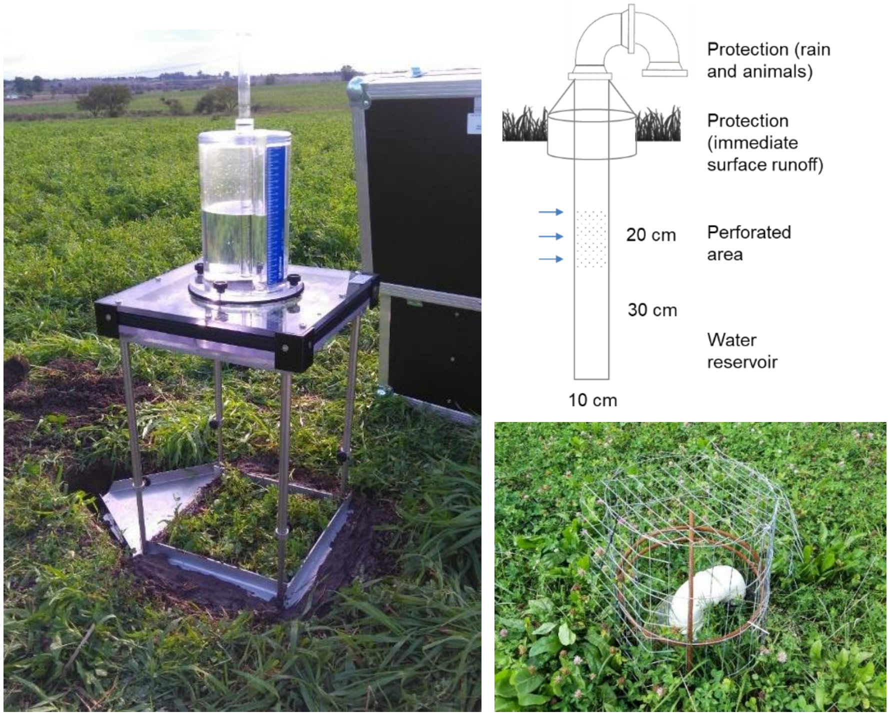
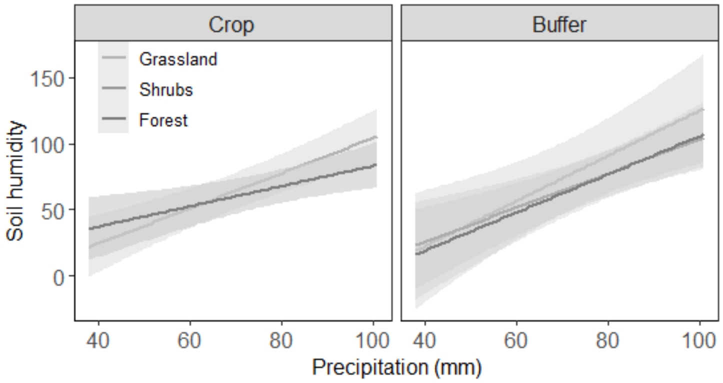


Figure A.1. Left: Picture of the rainfall simulator in the field. Roght: A schematic diagram of the design of the subsurface runoff water collectors with the detail of the dimensions of each section and an image of a collector installed in the field inside a cultivated plot.



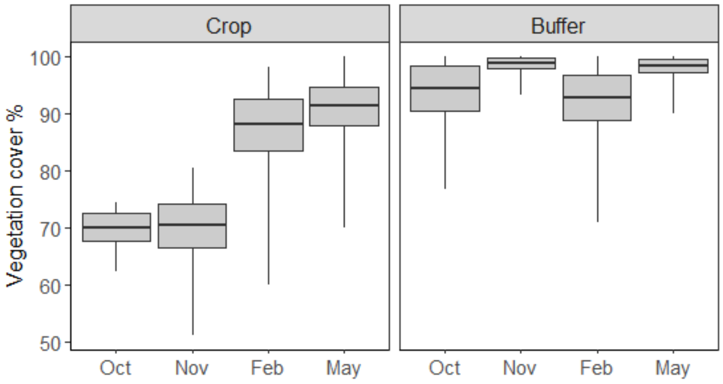


Figure A.2. Upper panels: Soil humidity (%) vs. accumulated precipitation (mm) in each event for the three crops located uphill of buffers (left), and the three buffers: grassland, shrubland, and forest (right). Lower panels: Plot vegetation cover (%) in the four sampling dates when simulations were performed for crops and buffers, grouped regardless of site. Mean values, standard errors (boxes) and minimum and maximum values (whiskers) are shown.

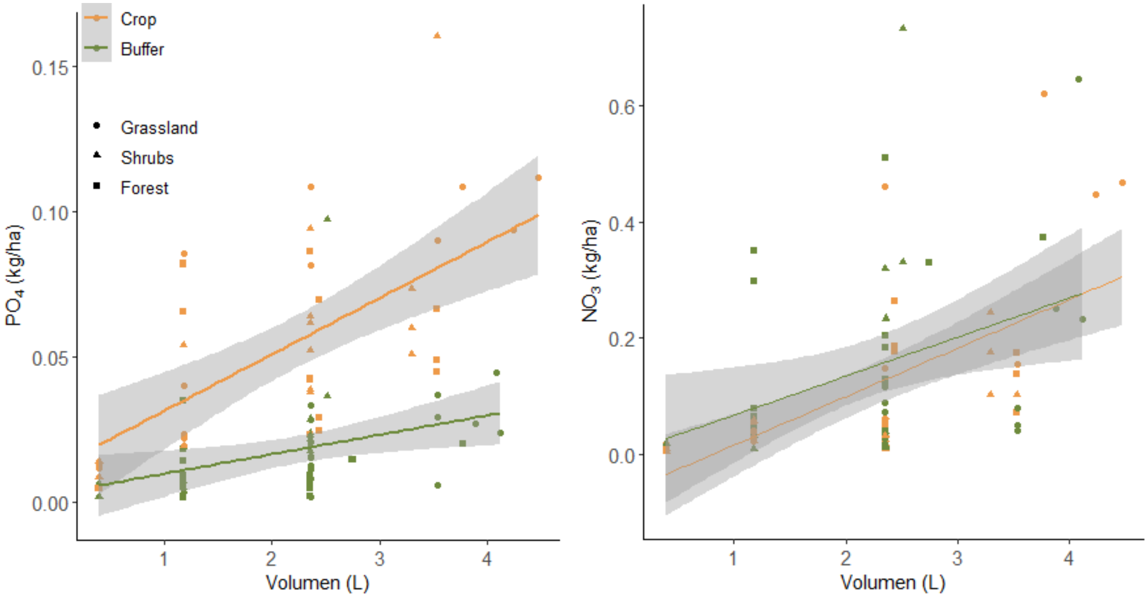


Figure A.3. Relationship between PO_4 and NO_3 load (kg/ha) with subsurface runoff water collected (L) in crops (orange) and buffer (green).

Table A.1. Summary of the eight storm events sampled, indicating type of sample collected on each date (surface or subsurface runoff water), accumulated rainfall (mm), duration of the event (days), number of previous days without rain > 10 mm, and average air temperature (°C), relative humidity (%) and wind velocity (km/h) during the week before sampling.

						Week before sampling		
Date	Type of sample		Accumulated rainfall (mm)	Length of event (days)	Previous days without rain >10 mm	Air T (°C)	Relative humidity (%)	Wind (km/h)
Aug-18		Subsurf	46	2	5	11.63	78.8	15.6
Sep-18		Subsurf	19	1	4	18.98	72.9	15.4
Oct-18	Surf	Subsurf	97	2	7	15.88	78.6	17.9
Nov-18	Surf	Subsurf	101	4	28	23.57	69.9	21.1
Jan-19		Subsurf	33	4	8	28.27	75.8	12.9
Feb-19	Surf	Subsurf	38	2	9	24.72	70.8	10.8
May-19	Surf	Subsurf	75	1	3	15.04	85.8	13.1
Jul-19		Subsurf	56	2	13	10.65	86.9	12.8

Table A.2. Soil characteristics of the crops and three buffer types: grassland, shrubland and forest. Conductivity (C, cm/h), organic carbon (%), clay, sand, and silt (%), organic matter (OM) and apparent density (AD) were measured at two depths: 0 - 5 and 5 - 10 cm. Phosphorus (Bray-P, ppm) and nitrogen (TN, %) were measured at 0 - 2.5, 2.5 - 7.5 and 7.5 - 15 cm.

Zone	z (cm)	C (cm/h)	Org C %	Clay	Sand	Silt	OM	AD	z (cm)	Bray-P (ppm)	TN %
Crop	0 - 5	1.17	3.405	24.8	21.2	54	5.87	1.54	0 - 2.5	25.5	0.42
	5 - 10	0.29	2.26	20	19.9	60.1	3.9	1.49	2.5 - 7.5	8.5	0.275
									7.5 - 15	5.5	0.205
Grassland	0 - 5	0.94	4.18	20	19.8	60.2	7.21	1.46	0 - 2.5	18	0.4
	5 - 10	0.56	2.28	20	19.7	60.3	3.93	1.56	2.5 - 7.5	7	0.19
									7.5 - 15	3	0.14
Shrubland	0 - 5	1.83	3.59	22.4	32.2	45.4	6.19	1.52	0 - 2.5	25	0.39
	5 - 10	2.68	3.06	22.4	30.4	47.2	5.28	1.31	2.5 - 7.5	12	0.3
									7.5 - 15	5	0.21
Forest	0 - 5	21.32	4.66	20	31.3	48.7	8.03	1.27	0 - 2.5	13	0.57
	5 - 10	17.23	3.44	22.4	30.2	47.4	5.93	1.26	2.5 - 7.5	7	0.35
									7.5 - 15	4	0.24

Table A.3. Results of two-way ANOVA testing main effects of factors: site (S), zone (Z: buffer vs crops) and depth, and their interactions (S*Z), on Phosphorus (Bray-P, ppm) and nitrogen (TN, %) measured at 0 - 2.5, 2.5 - 7.5 and 7.5 - 15 cm, and conductivity (C, cm/h), organic carbon (%), clay, sand, and silt (%), organic matter (OM) and apparent density (AD) measured at two depths: 0 - 5 and 5 - 10 cm. Only significant interactions of factors are shown.

	Site			Zone			Depth			S*Z		
	F	d.f.	p	F	d.f.	p	F	d.f.	p	F	d.f.	p
Bray-P			ns			ns	37.9	12	***			
TN %	4.12	12	*			ns	16.86	12	***			
Org C %	10.95	5	*	25.35	5	**	90.34	5	***			
C (cm/h)	10.45	35	***	11.79	35	**			ns	9.41	35	***
AP	22.92	47	***	8.19	47	**	5.18	47	*	3.52	47	*
Clay			ns			ns			ns			ns
Sand	35.87	6	***	24.83	6	**			ns	8.85	6	*
Silt	16.39	5	**	8.51	5	*			ns	7.4	5	*

Statistical results of ANOVA tests are shown, indicating F-values and degrees of freedom (d.f.) ns not significant $p>0.05$; $*p<0.05$; $**p<0.01$; $***p<0.001$

ARTICULO 2

Metodología para el monitoreo subsuperficial de aportes de nutrientes a sistemas hídricos, Embalse de Paso Severino, Uruguay

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Resumen: Evaluar las variaciones temporales y características del flujode agua dentro del suelo es relevante, especialmente a escala de cuenca, para identificar los mecanismos de generaciónde escorrentía y vías de transporte de nutrientes y contaminantes hacia los cuerposde agua superficiales. En zonas agrícolas, los manejos del suelo alteran las dinámicas naturales de los procesos hidrológicos y biogeoquímicos en sus primeras capas. El monitoreo del agua subsuperficial es complejo, ya que su flujo no es uniforme, y muchas de las técnicas permiten caracterizar dicho líquido sólo en términos cuantitativos. En este estudio, por su parte, se propuso una metodología para la obtención de muestras de agua subsuperficial, que permite captar y almacenar agua moviéndose enel suelo a una profundidad específica deinterés. Para ello, se diseñaron colectores cilíndricos plásticos con un área perforada, por donde ingresa el agua, y un sector de almacenaje. Se realizaron protecciones en dichos colectores para evitar el ingreso directo del agua de lluvia y del agua infiltrada verticalmente desde la superficieen el área inmediata a los mismos. La metodología fue puesta a prueba en predios productivos adyacentes al Embalse de Paso Severino (34°12´12´´S, 56°18´10´´O, Florida, Uruguay), donde permitió registrar diferencias en la concentración de nutrientes del agua subsuperficial entre zonas agrícolas y sus zonas buffer adyacentes. Además de efectivos, los dispositivos resultaronser de bajo costo y fácil construcción, por consiguiente, al realizar los ajustes específicos para cada suelo, el uso generalizado de estos dispositivos parece muy promisorio.

Palabras clave: escorrentía, contaminantes, zona de amortiguación, colectores.

Abstract: Assessing the temporal variation and characteristics of the water flow within the soil is key, especially at the basin scale, to identify the mechanisms of runoff generation and transport pathways of nutrients and pollutants to surface water bodies. In agricultural areas, different soil management may modify the natural dynamics of hydrological and biogeochemical processes occurring the first layers of the soil. The monitoring of subsurface water is complex since its flow is not uniform, and many of the techniques allow a quantitative-only characterization. In this study, we propose a methodology for obtaining subsurface water

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Recibido: Marzo 24, 2020 /Aceptado: Agosto 15, 2020

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samples, which allows to collect and store water moving in the soil at a specific depth of interest. For this, plastic cylindrical collectors were designed with a perforated area where water can enter, and a storage sector at the bottom. The direct inflow of rainwater to the pipes and of water infiltrating vertically from the surface in the immediate area was prevented by outside protection. The methodology was tested in fields bordering the Paso Severino Reservoir (34°12´12´´S, 56°18´10´´O, Florida, Uruguay), and it allowed us to record differences in nutrient concentrations in subsurface water between agricultural areas and their adjacent buffer zones. In addition to being effective, their low cost and easy construction, after making specific adjustments for each type of soil, suggest the widespread use of these devices is very promising.

Keywords: runoff, contaminants, buffer zone, collectors.

INTRODUCCIÓN

El movimiento horizontal del agua subsuperficial dentro del suelo es un componente importante del balance hídrico (Kim, & Mohanty, 2016); su flujo influye sobre la escorrentía superficial a nivel de cuenca, el sustento de caudales basales de cuerpos de agua, la reposición de la humedad del suelo, el transporte de sedimentos y nutrientes, entre otros procesos. Sin embargo, en general, se desconocen muchos aspectos de su generación, rutas de flujo, tiempo de residencia en el suelo, así como de los procesos biogeoquímicos asociados. La ocurrencia de flujo subsuperficial y su magnitud están controlados por múltiples factores, que involucran características de las precipitaciones, del suelo y la topografía, de la vegetación y de los usos (Hu, & Li, 2019), los que también afectan el contenido y disponibilidad del nitrógeno y fósforo en el agua.

El monitoreo del agua subsuperficial es complejo debido a que su movimiento no es uniforme y las técnicas apropiadas para su detección y caracterización no son sencillas. Además, los estudios empíricos realizados hasta el momento han variado enormemente en la nomenclatura, enfoques experimentales y escalas espaciales y temporales de análisis (*Chifflard*, *P*., et al., 2019). Hay ciertas técnicas de modelado que se han perfeccionado recientemente (Verrot et al. 2019), pero que no buscan obtener muestras, sino describir el flujo subsuperficial en términos cuantitativos.

Los procesos hidrológicos y biogeoquímicos en las primeras capas del suelo son particularmente sensibles en regiones agrícolas, por el efecto directo del uso y manejo del suelo, donde los sistemas de labranza, el tráfico de maquinaria y los períodos de suelo desnudo modifican la variabilidad temporal y espacial del contenido hídrico (Boizard et al., 2013). El proceso de eutrofización generalizado que se registra en los ecosistemas acuáticos superficiales del país (Bonilla et al., 2015), asociado por lo común al aporte difuso de nutrientes desde la agricultura (Goyenola et al., 2015), vuelve relevante entender su transporte en el agua subsuperficial que, al final, alcanza los cuerpos de agua superficiales. Los estudios más frecuentes a nivel mundial se centran en agua subterránea a través de piezómetros (Aguiar et al., 2015), pero los estudios sobre el agua subsuperficial son poco frecuentes.

El objetivo de este trabajo fue describir el diseño y los resultados principales de dispositivos para captar y almacenar a una profundidad específica el agua subsuperficial del suelo, proveniente ésta de sitios con distintos usos, para caracterizar sus propiedades físico-químicas.

DESCRIPCIÓN DEL ÁREA DE ESTUDIO

Los dispositivos de colecta de agua subsuperficial fueron puestos a prueba en predios productivos adyacentes al Embalse de Paso Severino (34°12´12´´S, 56°18´10´´O), construido sobre el Río Santa Lucía Chico, Florida, Uruguay. Este embalse es la principal reserva de agua dulce para potabilización del Uruguay; proporciona agua potable a casi 60% de la población del país. La intensificación de las actividades productivas en la cuenca ha comprometido seriamente la calidad del agua: se ha estimado que 80% de la contaminación por nutrientes se debe a fuentes difusas provenientes de actividades agrícolas (Manta et al., 2013). A su vez, la producción lechera se destaca como una de las actividades productivas que más influye en el aporte de nutrientes en esta cuenca (Pacheco et al., 2012; Chalar et al., 2017).

En un estudio reciente de Aubriot y colaboradores (2017), donde se analizó una serie temporal desde el 2004 al 2016, se encontró un aumento sostenido en la concentración de fósforo total (PT) en el agua del embalse, siempre con valores por encima de la normativa vigente. El embalse ha sido clasificado como hipertrófico, de acuerdo con la concentración promedio de PT (Arocena et al., 2008, 2012). En este estudio, se eligieron tres predios cultivados (pasturas implantadas), con pendientes similares y que tuviesen diferente tipo de vegetación *buffer* pendiente abajo, adyacente al espejo de agua. Los tres tipos de vegetación *buffer* fueron: dominancia de gramíneas (campo restablecido), dominancia de arbustos (arbustal) y bosque nativo establecido (bosque). En cada predio se instalaron colectores de agua subsuperficial en la zona cultivada y en la vegetación *buffer*.

METODOLOGÍA

Los dispositivos construidos para colectar agua subsuperficial consistieron en tubos de PVC de 11 cm de diámetro, sellados en el fondo con una tapa del mismo material y con un tramo de 20 cm de longitud con perforaciones pequeñas (2 mm) cada cm. Para su instalación se hizo una perforación con taladro de suelo de 10 cm de diámetro, de manera que el tubo de PVC ingresara ajustadamente. La profundidad a la que se enterró el tubo y en la que se ubicó la sección con perforaciones se determinó según las características del suelo, buscando representar el horizonte B. En la parte superior de cada colector se instalaron dos codos (figura 1), que permitieron el intercambio de aire e impidieron la entrada de agua de lluvia; fueron cubiertos con una malla para evitar el ingreso de pequeños animales. En la superficie del suelo y alrededor del colector, se colocó un cilindro de PVC de 20 cm de diámetro, insertado en el suelo 5 cm y cubierto con nylon, para evitar el ingreso de agua en la superficie inmediata al colector (figura 1).

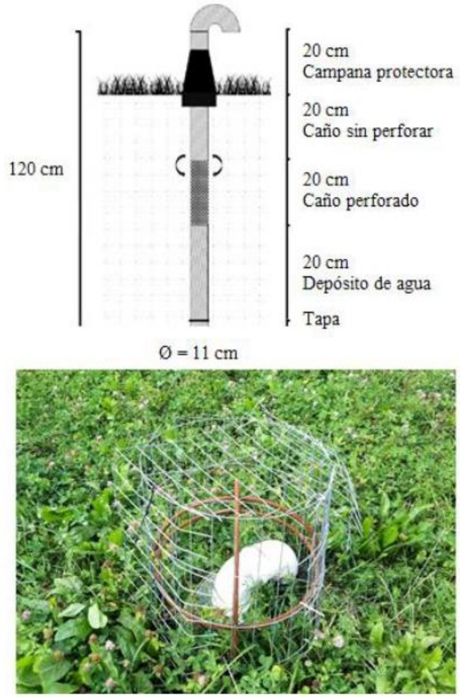

Figura 1. Arriba: esquema del diseño de los colectores de agua con el detalle de las dimensiones de cada tramo. Abajo: imagen de un colector instalado dentro de un predio cultivado.

Con este diseño, el agua de escorrentía superficial en un evento de lluvia no puede ingresar, mientras que el agua que circula por el suelo entre una profundidad aproximada de 20 a 40 cm puede ingresar por los poros del tubo, siendo almacenada en el fondo del mismo hasta el momento de su colecta (capacidad del reservorio inferior de hasta 2.4 L de agua). Alrededor de los colectores, se colocaron jaulas con malla metálica para proteger aquellos del ganado y otros animales.

Los colectores se dispusieron en transectas perpendiculares a la línea de costa del embalse. En cada uno de los sitios se instalaron tres colectores en el predio con cultivos y tres en las zonas *buffer* (i.e. campo restablecido, arbustal y bosque), con un total de 18 colectores (figura 2).

Figura 2. Arriba: esquema del diseño experimental, muestrala ubicación de los colectores de agua subsuperficial (círculos grises) dentro de los cultivos y los diferentes tipos de vegetación *buffer*, así como su ubicación respecto al embalse. Abajo: imágenes satelitales del sitio de estudio y los predios seleccionados.

La colecta de agua se realizó dentro de las primeras 24 a 48 horas luego de eventos de lluvia seleccionados de entre 20 a 100 mm, dependiendo de la estación del año, durante el período julio 2018-junio 2019. En campo, se retiró la protección de cada colector y se midió la temperatura con sonda multiparámetro (MACRO 900, Palintest Ltd.), % de oxígeno disuelto, pH, conductividad y profundidad del agua colectada, esto último para realizar cálculos de volumen. Las muestras se extrajeron con una bomba conectada a una batería de 12V, y fueron mantenidas en frío hasta su arribo al laboratorio. Al finalizar cada muestreo, los colectores fueron lavados: se pasó un cepillo por los poros y se volvieron a ensamblar. En laboratorio, el agua se depuró con filtros GF/C y se determinó la concentración de fosfato, nitrato y amonio, según metodologías estandarizadas (APHA, 1998).

La carga de nutrientes fue calculada multiplicando la concentración en el agua colectada por el volumen de agua almacenada dentro de los colectores. La superficie de colecta de los dispositivos fue estimada en 0.1 m², teniendo en cuenta las características del suelo y el movimiento del agua dentro del mismo.

RESULTADOS

Todos los colectores juntaron agua, aunque con variabilidad entre colectores adyacentes, en especial cuando las precipitaciones fueron bajas. Sin embargo, en la primera instalación dentro de uno de los sitios, los dispositivos en la zona del cultivo no colectaron agua, aun cuando fueron cambiados de lugar dentro del mismo cultivo. Finalmente, se cambió de predio, donde funcionaron con normalidad. Al analizar el suelo de dicho sitio, se observó un severo estado de degradación y compactación subsuperficial, donde la conductividad hidráulica en suelo saturado estimada en laboratorio (datos no presentados) fue < 0.01 cm/h.

El diseño de los dispositivos resultó ser efectivo en la colecta de agua subsuperficial. Los colectores fueron de simple construcción y de bajo costo, además de fáciles de trasladar e instalar en el campo. La protección de los colectores hizo posible evitar roturas por causa del ganado. El tiempo de construcción de cada dispositivo fue de un aproximado de 2 horas, mientras que la instalación en campo llevó 2 horas exactas. Los materiales utilizados son de uso doméstico frecuente y, por lo tanto, muy accesibles tanto logística como económicamente.

En todos los eventos de precipitaciones mayores a 20 mm se pudieron obtener muestras de agua. El volumen colectado varió entre 0.4 y 3.5 L de agua captada por el colector, lo cual resultó suficiente para los análisis previstos. El contenido de sedimentos no se consideró en los análisis, dado que éstos no se mueven dentro del suelo a la escala temporal analizada. La extracción del agua almacenada resultó efectiva y sencilla, siempre y cuando la precipitación fuera suficiente para colectar volumen.

Al separar las lluvias muestreadas en dos grandes categorías, i.e., de menos y más de 50 mm, observamos una tendencia: a mayor cantidad de lluvia, más agua subsuperficial almacenada en los colectores. Cuando el volumen de agua colectado se mantuvo por debajo de las perforaciones del tubo, el volumen colectado fue completo, es decir, que se colectó el total del volumen circulante. Sin embargo, cuando el volumen de agua alcanzó las perforaciones, es posible que haya ocurrido un flujo de entrada y salida entre el tubo y el suelo. Este flujo posible no fue estimado.

PARÁMETROS FISICOQUÍMICOS Y NUTRIENTES EN EL AGUA

A modo ilustrativo, la tabla 1 muestra los eventos de precipitación analizados y los parámetros fisicoquímicos del agua colectada. Las celdas sin dato corresponden a fallas técnicas de la sonda multiparámetro, o se deben a que el volumen de agua en los colectores no fue suficiente para su medición.

La concentración de NO_3 fue generalmente mayor en todos los casos, seguida de la de PO_4 (tabla 2). La concentración de PO_4 fue mayor en los sitios con agricultura que en las zonas buffer asociadas. Por el contrario, la concentración de NH_4 y NO_3 tendió a ser superior en la vegetación *buffer*, quizá asociada al mayor contenido de materia orgánica del suelo (datos no presentados).

Tabla 1. *Descripción de los eventos de lluvia en los que se colectó agua subsuperficial. Se indica la precipitación (PP) de cada evento y las características fisicoquímicas del agua colectada (temperatura (T), % saturación de oxígeno disuelto (%OD), y pH)*

Fecha	**PP (mm)**	**T (°C)**	**%OD**	**pH**	**Vol. (L)**
Ago/18	46	-	-	-	2.35
Set/18	20	24.3±1.3	-	7.1±0.1	0.65
Oct/18	97	16.3±0.6	-	6.5±0.4	2.45
Nov/18	101	20.5±1.4	45.1±30.1	5.5±0.7	2.65
Ene/19	33	25.6±1.4	43.1±10.5	6.8±1.0	1.42
Feb/19	38	22.3±0.6	33.1±7.6	6.9±0.4	1.69
May/19	75	17.3±0.8	-	6.5±0.3	1.78
Jul/19	56	-	-	-	3.10

Tabla 2. *Rango promedio de los distintos sitios (agrícola y buffer) para las concentraciones de PO_4, NH_4 y NO_3 en el agua subsuperficial*

Nutriente (µg/L)	**Agrícola**	***Buffer***
PO_4	230 - 331	104 - 123
NH_4	109 - 118	60 - 138
NO_3	321 - 692	413 - 852

CONCLUSIONES

Para nuestro sitio de estudio, la metodología evidenció valores inferiores de PO_4 en el agua subsuperficial de las zonas *buffer*, respecto a las zonas agrícolas asociadas. Para el nitrógeno no se encontró una relación clara entre los valores de las zonas agrícolas y las zonas riparias, aunque tendió a ser superior en la zona *buffer*.

Los dispositivos diseñados permitieron una colecta eficiente y precisa de agua subsuperficial de los horizontes A y B del suelo, lo que permitió una normal determinación de distintos parámetros fisicoquímicos y de nutrientes (nitrógeno y fósforo) disueltos. Los dispositivos permitieron la estimación simultánea de concentración y carga de nutrientes en el agua subsuperficial del suelo, aunque se precisan ajustes para la mejor estimación del volumen cuando el exceso de agua sobrepasa la capacidad de almacenamiento de los tubos. El uso generalizado de estos dispositivos parece muy promisorio, ya que fueron eficientes para colectar agua en suelos de baja a moderada conductividad hidráulica (Argiudoles Típicos). Se espera que, en suelos más permeables, estos dispositivos tengan capacidad de colectar sin dificultades. Si bien en los colectores se registró presencia de sedimentos (en baja magnitud), esto se debió al diseño del dispositivo, ya que los poros permiten el ingreso de suelo. Este aspecto debería mejorarse en el diseño de futuros estudios. Es posible que se requieran ajustes de acuerdo con la estructura y profundidad del horizonte de interés y también en función de la porosidad y granulometría del suelo, pudiendo ser necesario ajustar la densidad de los poros y la capacidad de almacenamiento (o implementar la remoción de agua mediante bombeo) en suelos arenosos. En futuros diseños se deberá aumentar la longitud del tubo para aumentar la capacidad de almacenamiento, o bien, tener un sistema de extracción y cuantificación del agua excedente para estimar con precisión el volumen circulante, aspectos que complejizan el dispositivo actual. A su vez, se deberá considerar la efectividad de los dispositivos en distintos suelos. El colector diseñado puede ser un dispositivo útil para entender mejor los procesos hidrológicos y biogeoquímicos, permitiendo recolectar agua en pasos secuenciados en el tiempo.

AGRADECIMIENTOS

Este trabajo pudo ser realizado gracias al Convenio entre DINAMA-MVOTMA y el CURE-UdelaR “Evaluación de la dinámica del fósforo en zonas *buffer* en el Embalse de Paso Severino y propuestas de alternativas de manejo para la reducción de los aportes difusos de este nutriente”. Agradecemos especialmente al Sr. Gerald Atrio por su valiosa ayuda en el campo.

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ARTICULO 3

Experimental warming and rainfall regimes lead to higher nutrient release and changes in Carbon fluxes from crop and buffer zone soils

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Abstract

Under a climate change scenario, mean temperatures are expected to increase and flood events to be more frequent, severe and less predictable. These climatic pressures will coexist with agriculture intensification, probably enhancing the already excessive transfer of nutrients to surface water bodies. One of the most frequent rehabilitation strategies to reduce nutrient losses and store carbon is the maintenance or re-establishment of riparian zones. However, if nutrient loading from the watershed is high, saturated buffer strips can act as sources rather than sinks, particularly during high flow events when soil is water saturated or flooded. Here, we experimentally mimicked water saturated and flooded riparian and agriculture environments by simulating a two-weeks light rain and a short-term heavy rain scenario, in combination with warming (two levels: control and elevated temperature), over crop and forest soils from a key watershed in Uruguay. We found both temperature and rain regime to affect nutrient release and CO_2 emissions, but was not possible to generalize about the outcomes of climatic variability, as they greatly depend on soil land use. If crop soil is waterlogged or flooded, a significant PO_4 exportation from soils occurs, and warming acts on top of that enhancing the release. In forest soils, such release happened only in the extreme case of flooding and with a much lower effect of warming. For nitrogen, the loads mobilized during the short-term flood were not high for water quality standards, but we detected changes in the nitrate and ammonium proportions due to flooding. For total nitrogen and nitrate, we registered an overall enhanced release under warming and from forest soil, while ammonium release was higher in forest but did not show an evident effect due warming. Regarding CO_2, we found a noticeable result in the switch from sink to source in forest soils under the scenario of warming and rewetting. Our experimental results suggest that warming and extreme precipitation will alter nitrogen cycle alterations and enhance the risk of freshwater eutrophication due to an increase mobilization of soil P from watersheds. In forest, the increase in soil-surface C release to the atmosphere with increasing temperature and water availability suggest that global warming will accelerate soil organic-carbon decomposition, and potentially drive positive feedback to future warming. The development of universal strategies to mitigate the negative impact of flooding under a warmer scenario could be complex, as responses depend on land use cover.

Key words: climate change, extreme rainfall, land use, nutrient release

1. Introduction

Diffuse pollution is a major threat to surface water bodies, and one of the main causes of aquatic ecosystem deterioration, due to eutrophication caused mainly by nutrient transfer from agricultural land (Kronvang *et al.,* 2007, Jabłońska *et al.,* 2020), causing a wide range of environmental, social and economic problems (Bender *et al.,* 2018). In addition to increased nutrient loads due to fertilization, agricultural land-use changes tend to increase runoff of precipitation and thus a higher volume of water reaches aquatic environments than from soils covered by grassland, shrubland or native forest (Aguiar *et al.,* 2015; Calvo *et al.,* under review). Under this scenario, one of the most frequent rehabilitation strategies to reduce the external load of nutrients is the maintenance or re-establishment of riparian zones, which act as buffers through the storage and purification of water.

Floodplains, where riparian areas are usually located, could proportionally be greater phosphorus (P) sources than upland soils depending on the amount of P stored in the soil, floodplain area and inundation period (Reavis & Haggard, 2016). If nutrient loading from the watershed is high, phosphorus and nitrogen immobilization and transformation rates via biological processes (i.e., microbial mineralization, denitrification and plant assimilation) can be insufficient and lead to an enrichment of soils with labile forms (Stutter *et al.,* 2012; Valkama *et al.,* 2019). The enhanced soil P cycling and increased soil P solubility compared to agricultural lands, can lead to a high risk of dissolved phosphorus leaching into water bodies (Stutter *et al.*, 2009; Stutter & Richards, 2012), even bigger if leaf litter is accumulated in the soil (Franklin *et al.*, 2020). Under certain circumstances nutrient saturated buffer strips can therefore act as sources rather than sinks, particularly during high flow events, ice thaw, and high bank erosion conditions (Dosskey *et al.*, 2010; Dodd & Sharpley, 2016; Vidon *et al.*, 2010). A higher frequency of cycles of flood and exposure in low lands could facilitate the decomposition and reduction of soil organic matter, which under normal conditions is effective in retaining P and N (Barret & Burke *et al.*, 2000; Wang *et al.*, 2021). This is especially relevant in riparian zones surrounding reservoirs, which have particular hydrology dynamics and a water regime that is frequently artificially regulated, together with climate variability and anthropogenic activities carried out in the catchment area (Ye *et al.*, 2019; Wang *et al.*, 2021).

For nitrogen, the removal in riparian zones is commonly attributed to both denitrification, which permanently removes N from the soil to the atmosphere, and plant uptake, where N is only temporarily retained and eventually returns to the available pool once mineralized (Hefting *et al.*, 2005). The balance between overall retention and environmental nutrient loss, are functions of hydrology and soil organic matter properties (Castellano *et al.*, 2013; Zhu *et al.*, 2018). Both waterlogging and flooding can result in nitrogen losses, reduced uptake and overall lower use efficiency of nutrients (Kaur *et al.*, 2019). Riparian areas that have not previously been flooded and likely have larger stock of nutrients that could be released upon flooding may have an exacerbated effect on both phosphorus and nitrogen release, with potential effects on nutrient cycling at the basin scale.

Under a climate change scenario, mean temperatures are expected to increase and flood events to be more frequent, severe and less predictable (IPCC, 2021). This is likely to have impacts on the terrestrial surroundings, as extreme flood events can result in large land areas with floodwater persisting even for several weeks or months (Najibi & Devineni, 2018). At higher temperatures, chemical and biological soil reactions are enhanced (van der Putten *et al.*, 2016). This is assumed to magnify the effects of extreme floods, potentially leading to higher nutrient release rates from flooded soils, with mobilization rates increasing with temperature (Tang *et al.*, 2016). Under warmer temperatures, microbially mediated redox reactions are enhanced, and thus P release to floodwater (Kumaragamage *et al.*, 2020). For sediment lakes, there is also evidence of enhanced phosphorus release with higher temperature (Jeppesen *et al.,* 2009; Wang *et al.,* 2013).

Greenhouse gas (GHG) emissions from soils may change in response to changing weather. Flooding may increase soil net GHG emissions, due to changes in soil redox potential and microbial activity (Hou *et al.,* 2000), while soil warming accelerates organic matter decomposition thus increasing soil-surface CO_2 release to the atmosphere (Davidson & Janssens, 2006). The combined impacts of flood and warming can lead to high gaseous C loss from grassland soils, partly due to plant senescence and large amounts of decaying material (Sánchez-Rodríguez *et al.,* 2019b). In crops, the outcomes of flooding emissions depend on agricultural practices regarding plant covering, fertilizers, organic residues, etc. (Sánchez-Rodríguez *et al.,* 2017). In reservoir sediments, the re-wetting after a drought leads to an initial release of gases accumulated in the pores during the dry period and latter an enhanced microbial decomposition of organic matter into CO_2 (Kosten *et al.,* 2018; Paranaiba *et al.,* 2020). CO_2 fluxes from drawdown areas can vary as a function of adjacent land cover types, with more CO_2 emissions near forestland than near grassland, likely because forest has more organic matter (Almeida *et al.,* 2019). In riparian soils, the elevated storage and availability of nutrients influence biological productivity and consequently might affect CO_2 fluxes (Petrone *et al.*, 2008), together with seasonal changes in biogeochemical conditions (Vidon *et al.,* 2014).

The timing and temporal variability of floods is primarily driven by climate, while land use and hydrological infrastructure would be more important influencing their magnitude. If offend flooded, riparian zones can contribute to water quality degradation and change carbon fluxes, thus limiting their long-term effectiveness. An improved understanding of biogeochemical mechanisms affecting nutrients' transport in riparian zones will help predict removal effectiveness under different future scenarios, as well as the occurrence of potential feedbacks with climate change due to differential behaviors as source or sink of carbon. In this study, we experimentally mimicked water saturated and flooded riparian and agriculture environments by simulating a two-weeks light rain and a short-term heavy rain scenario, in combination with a warming scenario, over crop and forest soils from a key watershed in Uruguay.

We hypothesize that water saturation and flooding alter microbial activity and the mechanisms acting and therefore expected changes in nutrient cycling, with more P released to water and modified nitrogen forms ratios. As temperature is a key regulator of biochemical reaction rates in soil, we hypothesize that warming shapes the outcome of flooding on soil nutrient release and carbon fluxes, by accelerating chemical and biological soil reactions and thus enhancing nutrient release and CO_2 emissions due to increased decomposition of organic matter. Further, we hypothesize that land use will strongly influence nutrient in soil superficial layers, in crops due to fertilization history and management practices, and in forest due to potential nutrient retention and enrichment. The specific concentrations and dynamics will shape the release of nutrients to soil and flood water, and also its interaction with climatic impacts.

2. Methods

2.1. Site, sampling and initial analyses

The soil samples were taken in winter from the Paso Severino Reservoir, located in the Santa Lucia Basin, Uruguay (34°12'12"S, 56°18'10"W). Climate is humid subtropical with hot summers and without dry season (according to the Köppen-Geiger climate classification), with annual average temperature of 17.0 °C and precipitation of around 1300 mm, based on the historical period 1961-1990 from the Uruguayan Meteorological Institute. The seasonal distribution of rainfall is highly variable, as well as the rain intensity among precipitation events, and long events are not uncommon. Soil water content depends on the precipitation-evaporation balance, normally storing water during the low evapotranspiration seasons when it becomes progressively wet and reaches saturation state (Silveira, 2000). The crop sampled was an artificial perennial ryegrass pasture. The native forest had a high density of large herbaceous plants, such as *Eryngium* sp. and trees such as *Allophylus edulis, Schinus longifolius,* and *Scutia buxifolia,* among other species. A high number of shoots of invasive species *Fraxinus americana* and *Gleditsia triacanthos* were also found.

Thirty-two intact corers of the first 15 cm of soil (6 cm diameter) from crop and native forest fields were extracted randomly by the direct push method, using transparent acrylic tubes. The bottom of the tubes was sealed with rubber material and tubes were transported in cold boxes to the lab. The corers were removed with their vegetation, which was scarce because of the season, and the superficial layer of litter. Extra samples of the upper 15 cm of the soil were taken to the lab to determine the concentration of N-NO_3 (ppm, specific ion method), P (Bray 1, spectrophotometric method), organic carbon (%, Walkley-

Black method) and Fe (ppm, atomic spectrophotometry method). Also, the initial humidity content was estimated by placing extra samples at 105 °C for three days, and organic matter by burning the dried samples at 500 °C for 2 hours.

2.2. Experimental design

Soil corers brought from the field were placed in wood structures inside climatic chambers to acclimate for 4 days and randomly assigned to experimental treatments. The design was completely factorial with 32 experimental microcosms in total, 2 cover soils: crop (C) and forest (F), 2 temperatures: control (C) and warming (W), 17.5 and 20.5 °C, and 2 rainfall regimes: a long-lasting light rain (LR) and long-lasting light + short-term heavy rain (HR), with 4 replicates of each treatment (Fig. 1). Our response variables were PO_4 concentration in soil water and TP, PO_4, TN, NO_3 and NH_4 concentrations in flood water. In addition, we measured CO_2 flux in all treatments during the different phases.

The tubes received low intensity light of approximately 80 lum/ft² (PAR) from fluorescent lamps with a light spectrum similar to that of sunlight. A Rhizon sampler (0.12-0.18 μm pore size; Rhizosphere Research Products, The Netherlands) was inserted into the middle of each soil corer at the beginning of the experiment to collect soil water throughout the experiment, minimizing the damage to soil structure (Seeberg-Elverfeldt *et al.*, 2005). All experimental units were placed at the same height to avoid temperature fluctuations and to homogenize the quantity of incident light, and a relative humidity of 70%. We randomly distributed the thirty-two soil corers between the two temperature treatments.

After this acclimation phase, on day 5 we started the simulated light rain in all the tubes, by carefully and gradually adding distilled water up to the soil surface, using a syringe connected to a tube to minimize soil disturbance. Here we define waterlogged soil as when soil pores are saturated with water but water does not exceed the soil surface (Kaur *et al.*, 2019). In the light rain treatment (LR), the soil remained waterlogged for 15 days, simulating a long and maintained period of light rainfall. Meanwhile, in the light + heavy rain treatment (HR), on day 9 we flooded the tubes with distilled water approximately 10 cm above soil surface. The tubes remained flooded for 5 days, simulating an episode of heavy rain. Then, water above the soil was carefully removed with a syringe and the soil remained waterlogged for five more days. On day 20, the tubes from all treatments stop receiving water and remained 4 more days (soil recovery phase) until the end of the experiment. Flood durations vary greatly in the literature from 24-hs to several months (e.g., Reavis & Haggard, 2016; Young & Ross, 2018), to mimic local conditions or study a particular mechanism. Here, we chose our water-saturated and flooded periods according to information gathered in the area (no published data).

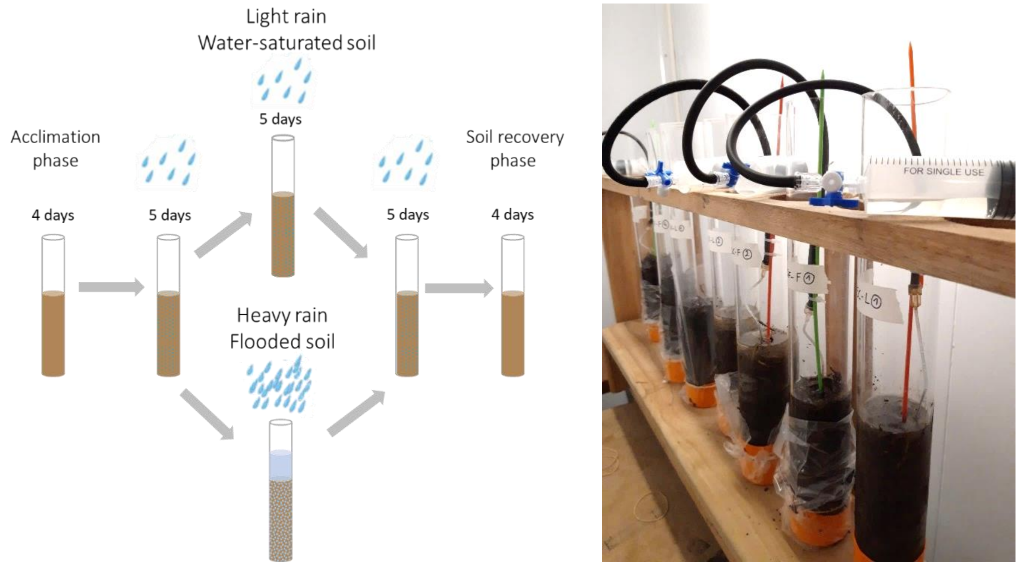

Figure 1. Schematic design of the experiment and the duration (days) of the periods: acclimation, light rain, heavy rain and recovery. Right: picture of the soil corers during the experiment.

We collected soil water every 4-5 days from an intermediate depth of the soil column with the Rhizon samplers connected with a 50-mL syringe, from both waterlogged and flooded corers. In every measurement, they were left till they have collected enough water for the analyses (~20 hs) and the samples were analyzed for phosphate (PO_4). Soil-water was measured in 2 or 3 random cores per treatment due to logistics associated to the experiment. Flood water (FW) was sampled only from the flooded treatment corers with a 50-ml syringe connected with a rubber tube from an intermediate depth of the water column. Flood water was analyzed for total phosphorus (TP), phosphate (PO_4), total nitrogen (TN), nitrate (NO_3) and ammonium (NH_4), according to standardized methodologies (APHA, 1998). Water lost due to evaporation and sampling was replaced, when necessary, by adding distilled water till soil was waterlogged (i.e., during light rain) and till water level was back to 10 cm over the soil surface when flooded (i.e., during heavy rain).

Every 2-3 days we did CO_2 measurements (environmental logger Senseair K33 ELG) in intervals of 30 seconds for 5 minutes in each tube. The sensor was placed in a plastic container attached to a rubber lid that hermetically sealed the tube, and precaution was taken in every measurement to wait till the air was homogeneous in both compartments to start recording. Diffusive fluxes were estimated based on the slope of the gas concentration versus time (Almeida *et al.*, 2016). A data quality control was applied based on R^2 values of linear fit for CO_2 measurements (above 0.70). Calculations with lower R^2 value were removed from the database. In addition, we continuously assessed light and temperature *in situ* with sensors set inside the chambers (Hobo Pendant MX-Temp, Onset), with recordings every 15 minutes. The study was carried out under a 12:12 day:night photoperiod. Dissolved oxygen (DO) concentration of floodwater was measured daily during the flood by inserting a probe (Oxyguard) midway between the soil surface and top of the water column. All tubes were left uncovered during the experiment to allow oxygen diffusion, thus mimicking field conditions as much as possible. After 23 days, the experiment was concluded and final samples of soil from each treatment were analyzed.

2.3. Data analysis

Differences in PO_4 soil-water concentration among treatments was assessed using three-way ANOVAs with 2 levels for soil cover, 2 levels for temperature and 2 levels for rain. Three-way ANOVAs with 2 levels for soil cover, 2 levels for temperature and 4 levels for time (the days when nutrients in flooded-water were sampled) were used to test the effect of the treatments on TP, PO_4, TN, NO_3 and NH_4 concentrations in flood water. In both cases Tukey's HSD function was applied for pairwise comparisons. Shapiro-Wilk tests were conducted to test for normality and Levene’s test for homoscedasticity.

For CO_2 analysis we defined period according to rain (acclimation, light rain, heavy rain, recovery). To test differences in CO_2 flux during each period between soil types, rainfall regimes and temperature treatments, we calculated the mean emission during the entire experimental period. Differences among treatments in mean emissions were assessed using three-way ANOVAs and Tukey's HSD function for pairwise comparisons. Shapiro-Wilk tests were conducted to test for normality and Levene’s test for homoscedasticity. All statistical analyses were conducted using the open-source software R (R Studio Team, 2018).

3. Results

3.1. Soil properties

Forest and crop soils exhibited different physico-chemical properties and drainage capacity, with forest soils being well drained while crop soils were poorly drained. In the initial characterization, crop soil doubled the values of nitrogen (N, ppm) and phosphorus (P Bray) content than forest soils, while forest presented higher iron concentration (Fe, ppm) and equal percentage of organic carbon (CO, %) (Table 1). The final samples showed high deviations in iron concentration in soils of both crop and forest. Soil organic matter (SOM) content was 6.62 % ± 0.69 in crops and 9.24 % ± 1.73 in forest (ANOVA, $F_{1,4}$ = 5.93, p=0.06). pH of the soil-water was always nearly neutral and did not differ between soils nor between temperature or rain treatments (p>0.05), and ranged between 6.0 and 7.1 in all treatments.

Table 1. Soil characteristics from crop and forest in both initial and final experimental time, for the treatments control, warming, light rain (LR), and light + heavy rain (HR): nitrogen ($N\text{-}NO_3$, ppm), phosphorus (P, Bray 1), organic matter (CO, %) and iron (Fe, ppm). na = not applicable.

	Initial		Final							
Soil	Crop	Forest	Crop				Forest			
Temp.	na	na	Control		Warming		Control		Warming	
Rain	na	na	LR	HR	LR	HR	LR	HR	LR	HR
$N\text{-}NO_3$	8.0 ± 1.7	3.7 ± 1.2	3.0 ± 0.0	4.0 ± 0.0	4.5 ± 2.1	3.5 ± 0.7	4.0 ± 1.4	3.5 ± 0.7	7.0 ± 5.6	3.0 ± 1.4
P	17.0 ± 3.6	8.7 ± 1.5	16.5 ± 2.1	11.5 ± 2.1	20.0 ± 1.4	13.5 ± 0.7	10.5 ± 0.7	10.0 ± 2.8	12.0 ± 2.8	12.5 ± 0.7
CO	3.0 ± 0.5	3.2 ± 0.2	3.04 ± 0.4	3.1 ± 0.2	2.9 ± 0.2	3.09 ± 0.04	3.3 ± 0.3	2.9 ± 0.4	3.3 ± 0.5	3.3 ± 0.2
Fe	189.3 ± 23.5	233.0 ± 14.7	280.5 ± 0.7	208.5 ± 60.1	182.0 ± 73.5	216.5 ± 28.9	219.5 ± 50.2	197.0 ± 79.2	201.5 ± 79.9	248.5 ± 62.9

3.2. PO_4 release in soil water

We did not find a clear generalizable effect on PO_4 release to soil-water with the climatic variables of warming or rainfall intensity. Instead, the effects depended strongly on soil type. In crops, PO_4 release to soil-water was higher under continuous waterlogged conditions (light rain treatment) than in the treatment adding a flood event. Also, warming enhanced PO_4 release to soil-water in the light rain scenario but not in the heavy rain one. Overall, we registered a higher release from crop soils, when compared to forest, in most simulated scenarios of temperature and rainfall. For forest, we registered an increase in PO_4 release from forest soil at control temperature after the flood.

In the light rain treatment (soil under waterlogged condition during the entire experiment) we registered the greatest values of PO_4 release to soil-water, higher than in the light + heavy rain treatment (average 161 ± 41 μg/L, versus 66 ± 50 μg/L in the heavy rain treatment). For crop soils, concentration was particularly higher at elevated temperature (average 179 ± 31 μg/L, versus 143 ± 42 μg/L at control), while in forest soils there was no significant effect of temperature (20 and 19.5 μg/L) (Fig. 1). In the forest soils, the highest release was found under flooding conditions and in the control than in the warming treatment. The mean concentration of PO_4 was significantly higher in crops than in forest, showing a substantial influence of the type of land use (Fig. 2, Table 2). Temperature produced significant differences, with higher mean release of PO_4 in the warmer treatment.

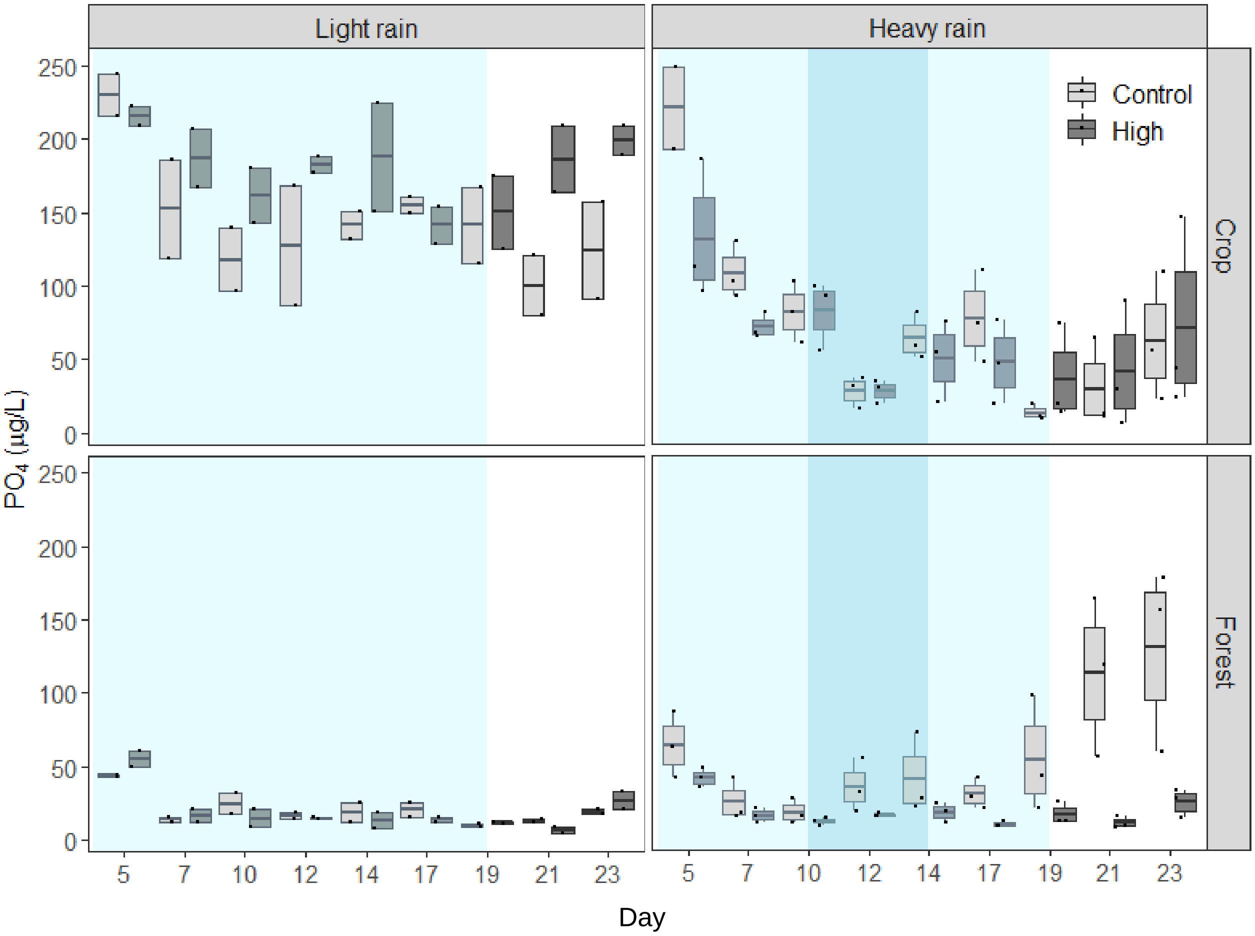

Figure 2. PO_4 concentration in soil water during light rain (left panels) and heavy rain (right panels) in soil water from crops (upper panels) and forest (bottom panels) soils under control (light gray) and high (dark grey) temperature. Mean values, standard deviation and minimum/maximum values are shown. Periods of light rain (lightest blue) and heavy rain (darkest blue) are shown in the background.

Table 2. Results of four-ways ANOVA testing main effects of factors: soil (crop vs forest), rain (light vs light + heavy), temperature (control vs warming), and their interactions, on PO_4 concentration (µg/L) in soil water. Statistically significant interactions are shown (Tukey test, $p<0.05$).

	d.f.	F	p			
Rain	172	13.73	***			
Temperature	172	7.68	**			
Soil	172	168.03	***			
Rain * Temperature	172	7.94	**	Heavy Rain-Control Light Rain-Warming	> >	Heavy Rain-Warming Heavy Rain-Warming
Rain * Soil	172	64.14	***	Heavy Rain-Crop Heavy Rain-Forest Light Rain-Crop Heavy Rain-Crop	> > > >	Light Rain-Crop Light Rain-Forest Light Rain-Forest Heavy Rain-Forest
Temperature * Soil	171	12.72	***	Control-Forest Control-Crop Warming-Crop	> > >	Warming-Forest Control-Forest Warming-Forest

Statistical results of ANOVA tests are shown, indicating respective F-values and degrees of freedom (d.f.) ns not significant $p>0.05$; $*p<0.05$; $**p<0.01$; $***p<0.001$

3.3. Flood-water

Flood water temperature were 17.9 and 20.2 ºC for the control and warming treatment respectively (Fig. S1). Dissolved oxygen was not different between temperature treatments, but it did differ between soil types, being lower in forest (ANOVA, $F_{1,60}=7.62$, $p<0.05$).

For both TP and PO_4 concentrations in the flood water the was a trend of higher concentrations under the warmer treatment. The effect of temperature was marginally significant in PO_4 for both crop (232 ± 30 vs 180 ± 27 µg/L in the control, mean ± se) and forest (average 151 ± 19 vs 123 ± 16 µg/L). TP and PO_4 were significantly higher in crop than in forest soils (TP: 249 ± 23 vs 188 ± 15 µg/L; PO_4: 206 ± 21 vs 137 ± 12 µg/L, crop and forest, respectively) (Fig. 3). For the crop soils submitted to elevated temperature both concentrations tended to decrease with time (Fig. 3). The proportion of PO_4 in TP was overall high in flood water, but significantly higher in crops (80.75%) than in forest (71.03%) (ANOVA, $F_{1,48}=12.57$, $p<0.001$), while no differences were recorded between temperatures and dates.

For total nitrogen, concentration in flood water was higher under warmer conditions only for crop (540 ± 92 vs 307 ± 30 µg/L, Tukey = 0.07) (Fig. 4). Under control temperature, the concentration increased with time, with the opposite trend in the soils submitted to elevated temperature (Table 3). Mean NO_3 concentration was higher under warming for crop (126 ± 29 vs 99 ± 20 µg/L in the control treatment) and forest (176 ± 24 vs 111 ± 15 µg/L in the control). Was also higher NO_3 in forest (144 ± 17 vs 113 ± 15 µg/L in crop) and decreased with time in all treatments (Fig. 4, Table 3). Meanwhile, NH_4 concentration under control temperature tended to increase with time and was higher in forest (Table 3). Under warming there was high variability in the concentration and no clear trend was observed (Fig. 4).

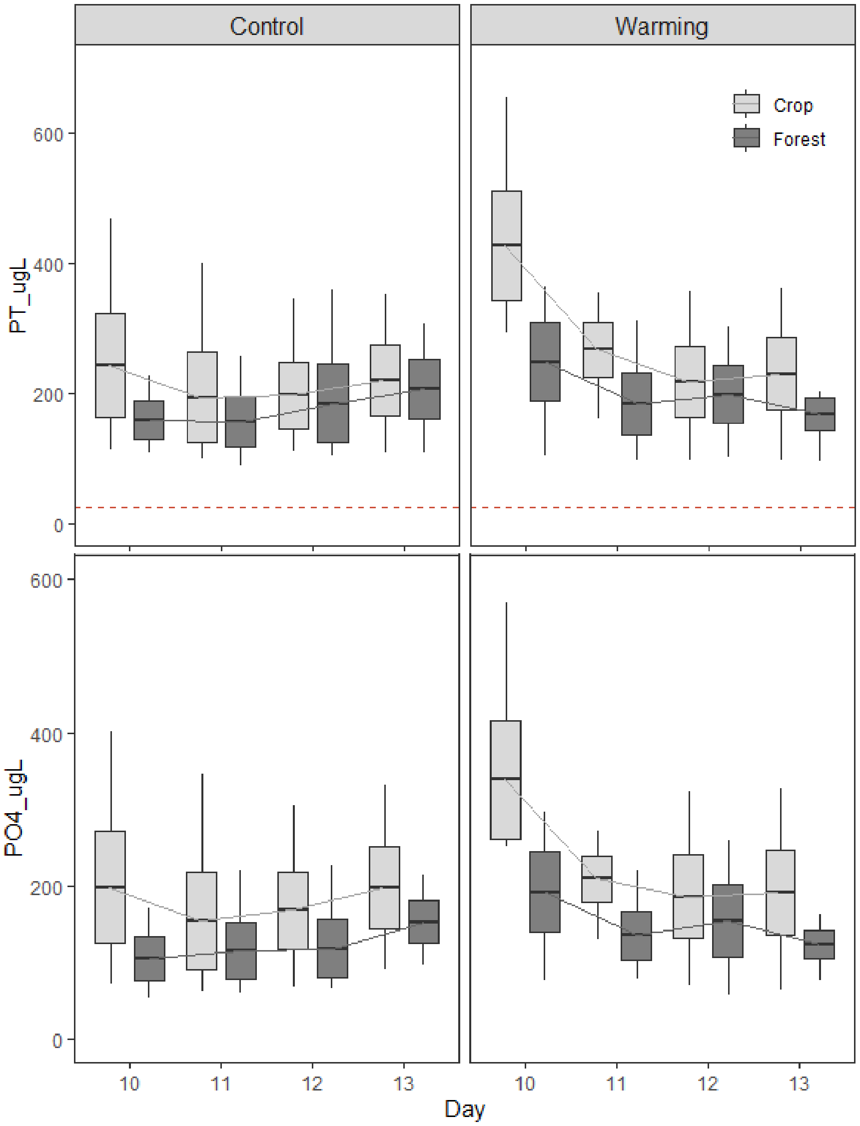

Figure 3. TP and PO_4 concentrations (µg/L) in flood water of crop and forest soils at control and high temperature. Mean values, standard errors (boxes) and minimum and maximum values (whiskers) are shown. The national maximum PT limit for freshwater systems (25 µg/L) is indicated with a red dash line.

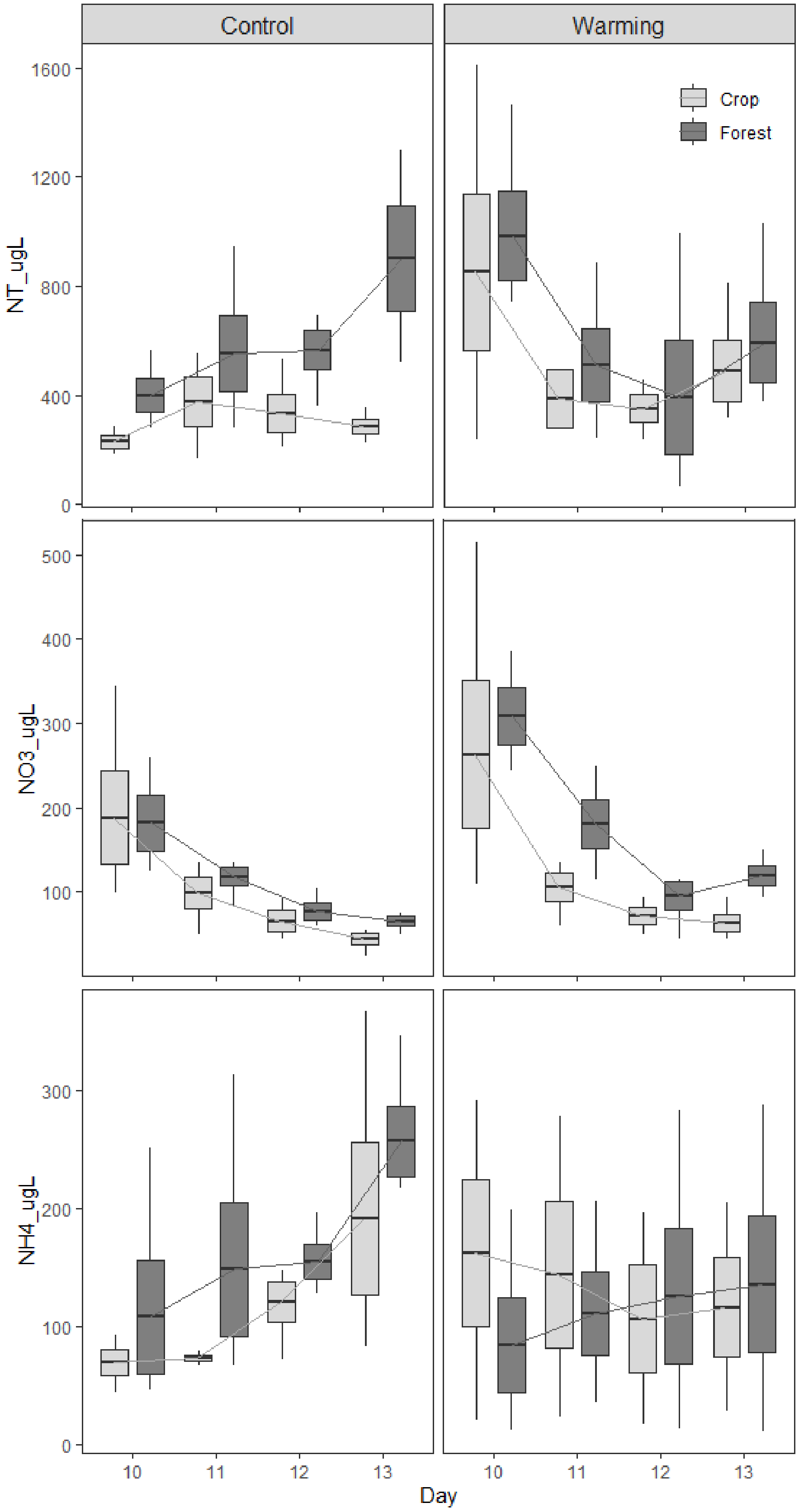

Figure 4. Total nitrogen (TN), nitrate (NO_3) and ammonium (NH_4) concentrations (µg/L) in flood water at control (left panels) and high (right panels) temperature and in both crop (light grey) and forest (dark grey) soils. Mean

values, standard errors (boxes) and minimum and maximum values (whiskers) are shown.

Table 3. Results of four-ways ANOVA testing main effects of factors: temperature (control vs warming), soil (crop vs forest), and time (4 sampling days), and their interactions, on TP, PO$_4$, TN, NO$_3$ and NH$_4$ concentration (µg/L) in flood water.

	TP		PO$_4$		TN		NO$_3$		NH$_4$	
	F	p	F	p	F	p	F	p	F	p
Temperature	2.93	0.08	3.02	0.06		ns	**12.11**	**		ns
Soil	**3.99**	*	**6.56**	*	**7.82**	**	**12.32**	***		ns
Time		ns		ns		ns	**31.78**	***		ns
Temperature * Soil		ns		ns	**4.76**	*		ns		ns
Temperature * Rain		ns		ns	**5.24**	**		ns		ns
Soil * Time		ns		ns		ns		ns		ns
Temperature * Soil * Time		ns		ns		ns		ns		ns

CO$_2$ flux

For rain regime, warming and land use we did not found a generalizable effect on CO$_2$. The only significant variable affecting CO$_2$ flux was the moment of measurement. For crops, we found at control temperature always positive values of flux (emitting CO$_2$). For forest, during the acclimation period, soils were emitting CO$_2$ at low temperatures and capturing at high temperature. Once rewetted, forest soil switched to CO$_2$ emission at high temperature (significant differences in ANOVA and Tukey tests).

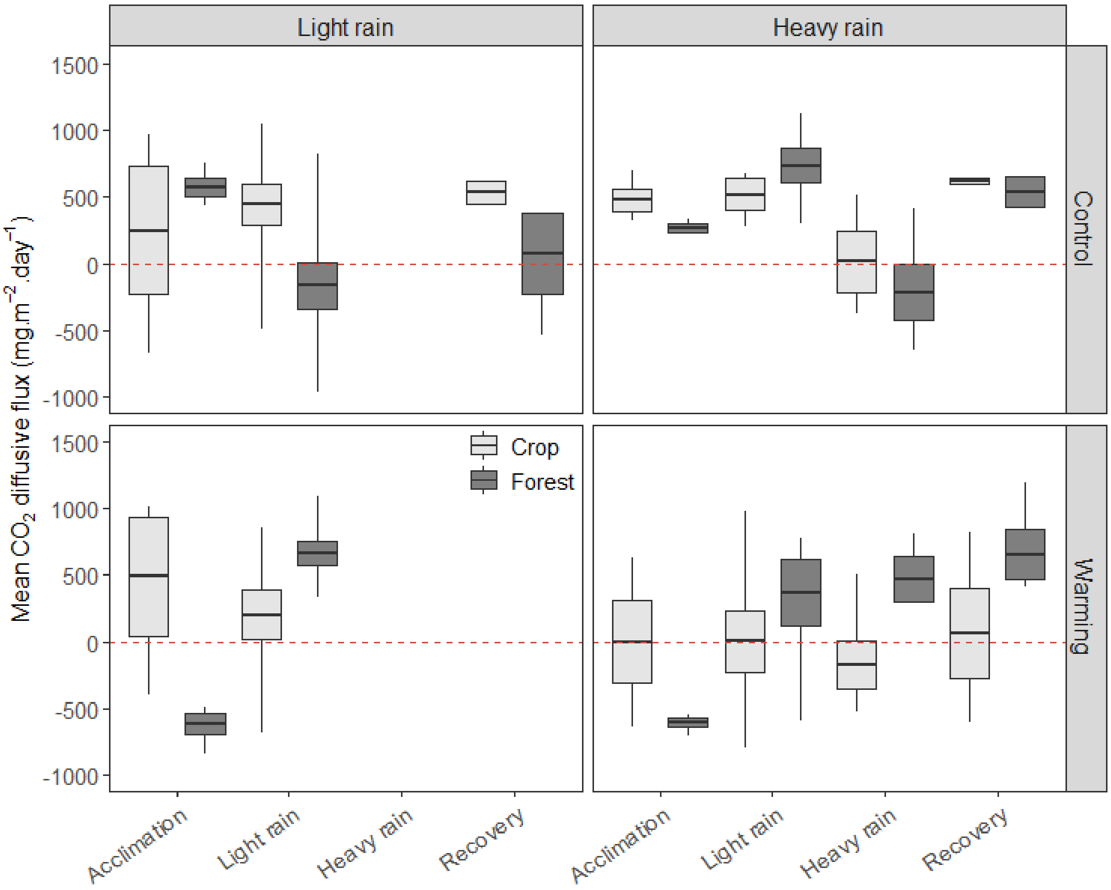

Figure 5. CO_2 flux during light rain (left panels) and extreme rain (right panels) at control (upper panels) and elevated (lower panels) temperature in crop (light gray) and forest (dark gray) soil. Mean values, standard errors (boxes), and minimum and maximum values (whiskers) are shown. 0 is indicated by a red dashed line. Values above 0 indicate CO_2 efflux or net emission to the atmosphere, while values below 0 represent CO_2 influx or net sink. Missing data is due to the quality control applied based R^2 values of linear fit for CO_2 measurements (data removed if $R^2 < 0.70$).

4. Discussion

Our results confirmed that soil saturation and flooding mobilize phosphorus and increased CO_2 emissions, and a potential synergistic effect of warming. However, we cannot generalize about the outcomes of climatic variability in temperature and rain regimes, as they greatly depend on soil land use. If crop soil is waterlogged or flooded, a significant PO_4 exportation occurs, and warming acts on top of that enhancing the release. In forest soils, such release happened only in the extreme case of flooding and with a much lower effect of warming. For nitrogen, the loads mobilized during the short-term flood were not high for water quality standards, but we detected changes in the nitrate and ammonium proportions due to flooding. For total nitrogen and nitrate, we registered an overall enhanced release under warming and from forest soil, while ammonium release was higher in forest but did not show an evident effect due warming. We registered a switch from sink of CO_2 to source in forest soils under warming and rewetting conditions.

Waterlogging and short-term inundation resulted in phosphate enrichment of soil and flooded water. Soil water PO_4 concentration was particularly high in crop soils, while in flood water, TP and PO_4 concentrations were high also in forest soils. The release from soils of both land uses far exceeded the limits allowed by national law for surface water bodies (TP: 25 µg/L, DINAMA). In this study, dissolved reactive P was the major fraction of P in floodwater, representing more than 70% in both crop and forest, and slightly higher in crops, as has been registered in previous experiments simulating flood events (Jeke & Zvomuya, 2018). The concentrations were generally much higher than those typically recorded in natural rivers, lakes and lagoons in a good conservation status (Goyenola *et al.,* 2015; Chalar *et al.,* 2017; Rodríguez-Gallego *et al.,* 2017). This reinforces the idea that periodical nutrient transportation from the terrestrial environment can affect water quality.

Although in different magnitudes, during both waterlogging and flooding, there was a reduction of oxygen in soil pores. The outcome of floodplain soil inundation typically consists of an initial increase in aerobic heterotrophy (with liberation of C, N and P from leaf litter and soils), potentially followed by a phase of anaerobic nutrient cycling processes if flooding continues (P release and denitrification) (Ponnamperuma, 1984; Baldwin & Mitchell, 2000). Reductive dissolution of Fe^{3+} oxides by microorganisms has been previously found to be the key process contributing to the increase of available P during the first stage of a flood event (Loeb *et al.,* 2008; Maranguit *et al.,* 2017; Concepcion *et al.,* 2021).

Total phosphorus release rates from flooded soil are significantly conditioned by land cover, with particular high release in bare or poorly vegetated lands (Peng *et al.,* 2019). This can explain the increased P released in crop soils; as anaerobic conditions are enhanced in compacted agricultural soils (and hence with a lower redox potential). In experimentally flooded agricultural lands with frequent fertilization history, most of the phosphorous released from soils occurred within the first 48 hours regardless of soil chemistry (Aldous *et al.,* 2007). Yin *et al.* (2018) study in an agricultural dominant reservoir estimated large amounts of P and N are released per year from the soil in the water-level fluctuation zone (i.e., the periodically submersed and exposed area by both anthropogenic regulation of water level and variability of rainfall). A decrease in pore water PO_4 concentrations with a consistent increase in floodwater, as partially registered in our experiment, might be evidencing a rapid diffusion from soil water to surface floodwater (Kumaragamage *et al.,* 2020), and its probable subsequent transport to freshwater bodies.

Besides crops, we found that in a scenario of flooding forest could also release high P concentrations, likely due to rapidly available P in soil. For vegetated buffers, biogeochemical processes transform the accumulated P into highly mobile and highly bioavailable dissolved P forms (Gu *et al.,* 2017), which can lead to fast P release rates (Maranguit *et al.,* 2017). Regardless of land use, our results are in agreement with the findings of Wang et al. (2021) who stated that exposure and flood cycles in the soils will increase the content of soil labile P and the linked risk of P release from the soil in the water-level fluctuation zone.

The quantity to be released upon flooded conditions depend largely on the availability of P in the soil. Reavis & Haggard (2016) registered in a 24-hours experiment with flooded corers, a correlation between soil P with SRP flux in the overlying water. In our experiment this was also the case, as initial soil P was higher in crop and both TP and PO_4 release in flood water were higher than in forest. Tang *et al.* (2016) also found that water extractable P was the best predictor of P mobilization rates upon short-term flooding. In a long-term experiment (75-days), Young & Ross (2018) found soluble reactive phosphorus in soil water to increase over time, while flood water tended to decrease its concentration. They registered an important mobilization of SRP from soil through pore water and a limited flood water release because of resorption (Young & Ross, 2018). In their 60-day experiment incubating soil from different land uses under anaerobic and flooded conditions, Maranguit et al. (2017) found the impacts of flooding to be rapid and not only important in terms of P release but also observed that soil P forms were modified to more available fractions. P stores are likely to be depleted more quickly due to preferentially mineralized over N from organic matter, which can lead to changes in N:P ratios and potentially have ecologically relevant impacts (Talbot *et al.,* 2021). In particular, organic P can be a substantial source of potentially-plant-available P by microbial mineralization when the soil undergoes drying and rewetting cycles (Chepkwony *et al.,* 2001; Richardson & Simpson, 2011).

Water level determines the availability of oxygen in soil, and hence is a key factor of nitrogen transformation (Noe *et al.,* 2013; Ye *et al.,* 2019). Hence, it was expected that the nitrogen cycle experienced significant alterations depending on the characteristics of the flood event. Nitrate concentration in flood water decreased over time for both land uses and both temperatures. Ammonium increased with time for both soils under the control temperature, and was expected to increase faster under warming (Sánchez-Rodríguez *et al.,* 2019b) but remained constant. Nitrification can be disrupted under anaerobic conditions, facilitating the accumulation of NH_4 in soil and its diffusion into the overlying floodwater (Nielsen *et al.,* 1996; Sánchez-Rodríguez *et al.,* 2018; Tan *et al.,* 2020). Ye *et al.* (2019) registered an increase in NH_4 in the riparian soil after submergence, through nitrogen mineralization. In agreement, Tomasek *et al.* (2019) registered increased denitrification rates in an experimental short-term flood, reducing nitrate transportation to water bodies.

In addition to the effects discussed above of increased water availability, and in more extreme cases of flooding, the increase in temperature can have interactive effects on soil characteristics and processes. In an experiment comparing soils with no previous history of flooding, warming was found to enhance the fast release of P into the soil and overlying water induced by flooding (Sánchez-Rodríguez *et al.*, 2019b). This synergistic effect of warming was also found in an experiment comparing extreme temperatures of 5 and 20°C (Loeb *et al.,* 2008). Our experiment found the same expected trend of enhanced release under warming, likely due to microbial reactions releasing P being influenced by temperature (Wang *et al.*, 2018).

Warming can also have profound effects on N cycling by a decrease in microbial immobilization and an enhanced mineralization, nitrification and denitrification in soils (Miller & Geisseler, 2018; Dai *et al.*, 2020). In our experiment, nitrate concentration decreased during the flood event regardless of land use and was higher in the warmer treatment. Both responses were expected as denitrification is strictly anaerobic and thus requires saturated soils (Pinay *et al.,* 2007), and nitrification rate increase with an increase in soil temperature, both in the surficial layer and deeper layers in the soil (Tan *et al.,* 2018). Although denitrification may be particularly sensitive to warming (Bonnett *et al.*, 2013), it was potentially masked and not observed here. The increasing accumulation of NH_4 at control temperature was expected, as anaerobic conditions during floods lead to NH_4 being the dominant N form in the soil (Gu & Yang, 2022). Meanwhile, in the warming treatment NH_4 concentration was not higher, contrary to expectations, as mineralization increases with temperature (Miller & Geisseler, 2018; Sánchez-Rodríguez *et al.*, 2019b).

This effect has been found in longer floods to be masked by a reduction in total microbial biomass (Sánchez-Rodríguez *et al.*, 2019b). Soil microbial biomass (not measured here) is typically reduced and community structure changed by long-term floodings (Sánchez-Rodríguez *et al.*, 2019a), and also by long term warming (Frey *et al.*, 2008).

Regarding land use, nitrogen cycling is strongly connected with soil microbial biomass and activity in forests (Bengtsson *et al.*, 2003), and also in crops, with an important role of management strategies in the latter (Burger & Jackson, 2003). In forest soil, nitrate concentration in flood water was higher than in crop regardless of temperature. Denitrification by microbial anaerobic respiration can be a relevant N sink in agricultural basins with fertilization history and susceptible to waterlogged conditions as the evaluated here (Soana *et al.*, 2022). Meanwhile, plant N uptake has been found to be high in forested buffer areas, but only temporal because N is available again once mineralized (Hefting *et al.*, 2005). This uptake by vegetation was negligible in our experiment.

Soil temperature and soil water content are the main abiotic factors controlling soil CO_2 concentration (Maier *et al.*, 2011), so it becomes relevant that low to moderate sustained temperature increases can alter the dynamics of soil water storage (Condon *et al.*, 2020). CO_2 emissions from continuously flooded crops have been found to be lower compared to systems with intermittent access to water (Sapkota *et al.*, 2020), or even negatively correlated with soil water content in a long-term simulated flood (Xu *et al.*, 2021). Poblador *et al.* (2017) measured riparian soil fluxes of CO_2 over a year, finding that water content was a primarily control of the temporal patterns of CO_2 emissions and registered a release peak after spring due to conditions of soil water content and temperature that enhanced respiration. Here, we found a noticeable result in the switch from sink of CO_2 to source in forest soils under the scenario of warming and rewetting. According to our results, climate change would promote higher emission of CO_2 to the atmosphere from terrestrial soils enriched with organic matter, in this case forests. In the same direction, Welsh *et al.* (2019) analyzed storm effects in riparian zone of an agricultural stream and found that a high intensity, short duration storm of low magnitude, following dry antecedent conditions, led to release of CO_2 across the riparian zone and low SRP removal (Welsh *et al.*, 2019). A high C loss (CO_2 and CH_4) when flooded and at elevated temperature can be partially consequence of large amounts of decomposition of plant material (Sánchez-Rodríguez *et al.*, 2019b). In our experiment this would be basically roots and leaves litter, since there was almost no aerial vegetation. This pattern of peak emissions upon rewetting after a dry period has also been registered in reservoir sediments after extreme droughts (Kosten *et al.,* 2018). In forest, the increase in soil-surface C release to the atmosphere with increasing temperature and water availability suggest that global warming will accelerate soil organic-carbon decomposition, and potentially drive positive feedback to future warming (Giardina *et al.*, 2014; Soong *et al.*, 2021). CH_4 and N_2O emission from riparian forest soil increase with flooding (Schindler *et al.,* 2020), so future studies should also measure them to get a complete picture of the role of riparian soils in potential feedbacks with climate change.

5. Conclusions

Our results suggest that the temporal waterlogging or inundation of agricultural floodplains will lead to increased nutrient mobilization, particularly phosphate, with the release enhanced by warming. Nutrient losses could potentially contribute to eutrophication of adjacent freshwater systems and jeopardize their quality, or even produce the accumulation of certain toxic elements in the soil (review in Kaur *et al.,* 2020). This could be exacerbated depending on soil moisture, as drier antecedent conditions are linked to greater losses of total and dissolved phosphorus once the soil is rewetted (Cassidy *et al.,* 2017; Sun *et al.*, 2017; Khan *et al.,* 2022). Prolonged inundation, such as in near-shore or lowland areas, increases the chances of soil P release and decreases P saturation (Wang *et al.*, 2020; Wang *et al.*, 2021).

The high nutrient retention effectiveness of the forest buffer zone in this reservoir under typical conditions (i.e., not waterlogged or flooded) was previously demonstrated (Calvo *et al.,* under review). In a waterlogging scenario, the release of phosphate was substantially higher from crop soils, and therefore, the establishment of buffer vegetation in the water-level fluctuation zone is an effective method of reducing nutrients inputs into the reservoir. However, in bigger rainfall events when the soil is flooded, the benefits of a buffer zone were not that evident, since phosphorus concentration in forest flooded water was similar to crop soil and nitrogen was generally higher in forest. Flood-resilient plants, such as anoxia-tolerant vegetation, can mitigate some of the negative impacts of flooding on soil functioning (Sánchez-Rodríguez *et al.,* 2019a).

The findings of this study are important to understand and predict the effects of (more frequent) floodings, acting simultaneously with warming and land use change, and to contribute to generate models to better predict P and N transport and C fluxes in riparian landscapes. The interaction between global changes such as rapid conversions in land use and warming, seem to have feedback through more CO_2 emissions. The results are relevant not only in flooding scenarios, but also for well-drained soils that are partly waterlogged by regular heavy rainfalls.

Funding: This study was supported CSIC-UDELAR (Initiation to Research grant, ID: 72, to CC), Uruguay. The study was partly supported also by DINACEA, Uruguay, and by ANII (POS_NAC_2016_1_130095). MM and CC are partly supported by CSIC I+D grant 511, ANII, and PEDECIBA.

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ARTICULO 4

Received: 6 August 2021 | Revised: 29 March 2022 | Accepted: 5 April 2022

DOI: 10.1111/fwb.13916

ORIGINAL ARTICLE

Freshwater Biology WILEY

Flow pulses shape periphyton differently according to local light and nutrient conditions in experimental lowland streams

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Funding information

This study was supported by Aarhus University, Denmark; ANII (POS_NAC_2016_1_130095), and CSIC-UDELAR (Initiation to Research grant, ID: 72, to CC), Uruguay. EJ also was supported by the TÜBITAK, BIDEB2232 program (118C250). MM and CC are partly supported by CSIC I + D grant 511, ANII and PEDECIBA. CA was supported by Doctoral INPhINTI - INCOMING program (LCF/BQ/DI20/11780004), La Caixa Foundation (ID 100010434).

Abstract

1. Streams generally are affected by multiple stressors acting at different timescales. Periphyton, often the most important primary producer in these ecosystems, may respond to short-term impacts as well as to different long-term environmental conditions with potentially various changes in community structure.
2. Here, we experimentally investigated the effects of sudden flow pulses on periphyton communities as a way to mimic extreme precipitation events in lowland streams that are predicted to occur more often with climate change in some regions. Using outdoor flumes, we allowed periphyton to colonise nutrient-diffusion substrates under two light conditions (50% shade and fully open) and nutrient availabilities (control, with access only to stream nutrients, and N–P-enriched) along a gradient in baseflows (0.43 to 2.17 L/s). After one month, we exposed the communities to a flow pulse (two-fold peak flow increase to simulate conditions of a potentially high disturbance) and analysed the responses of biomass and taxonomic composition.
3. Flow pulse promoted periphyton growth in the lowest range of the baseflow but led to biomass reduction in the highest range. Light was the second major driver of biomass accrual, whereas nutrient enrichment had a strong effect on community composition both before and after the pulse (i.e., diatom dominance vs. green algae dominance in scenarios without and with enrichment, respectively). In all treatments, the flow pulse promoted a higher taxonomic richness, suggesting a partial reset of the succession of the periphyton communities. However, independent of flow and resources, periphyton communities showed low ecological resistance against the pulse with changes in chlorophyll *a*, biovolume and taxonomic richness to the pulse.
4. We demonstrated that the effects of pulses on periphyton are similar in terms of biomass but varied strongly regarding composition depending on their initial structure, which is in turn mediated by the baseflow normally experienced by the systems, and on light and nutrient availability.
5. Our results highlight the importance of testing multiple stressors, such as an increase in extreme events, under a wide range of environmental conditions

(i.e., flow regime, trophic state, light availability). This approach allows us to detect potential interaction effects and non-linear responses, and highlights that the environmental settings ultimately determine the net effects of flow pulses on community structure and probably also on several important ecosystem processes.

KEYWORDS
discharge, disturbance, eutrophication, extreme streamflow events, light

1 | INTRODUCTION

Natural flow regimes control the composition, structure and dynamics of biological communities, and influence ecosystem processes such as nutrient uptake and transformation, organic matter processing, and ecosystem metabolism of running waters (Lytle & Poff, 2004; Poff, 2018). The flow regime varies geographically with climate, mainly through precipitation and temperature, and catchment conditions such as vegetation cover, soil and topography (Allan et al., 2021; Goyenola et al., 2015). Streams experience recurrent natural changes in flow, and the structure of the biological communities therefore is adapted to such disturbances (Guo et al., 2020; Wu et al., 2019), but sudden alterations in discharges can have vast effects on the ecological functioning and biodiversity of streams (Neif et al., 2017; Poff & Zimmerman, 2010; Wu et al., 2019). An increase in disturbance frequency can alter not only community composition, but also diversity, disproportionately affecting rare taxa (Haghkerdar et al., 2019).

Periphyton is a key food resource in stream food webs and a contributor to primary production and nutrient cycling, and it thus generally plays an important role in ecosystem functioning (Allan & Castillo, 2007; Larned, 2010). Besides, as a result of its rapid response to environmental changes, stream periphyton can serve as a bioindicator of water quality (Beck et al., 2019; Pacheco et al., 2022; Stevenson, 2014). The intensity and frequency of extreme short-term streamflow disturbances play an important role in shaping periphyton biomass and composition (Guo et al., 2020; Peterson & Stevenson, 1992), with quite different effects in different types of streams (Biggs et al., 1999). An increase in low flow velocity and turbulence may enhance periphyton growth because of a higher nutrient replenishment (Bækkelie et al., 2017; Horner et al., 1990; Larned et al., 2004; Whitford & Schumacher, 1961). However, a further increase may lead to biomass reduction as a consequence of detachment of organisms, scouring by sediments and movement of the substrates (Haddadchi et al., 2020; Hoyle et al., 2017; Luce et al., 2010). Furthermore, periphyton responses to flow are regulated by the availability of light and nutrients, and high flow may even enhance periphyton growth in conditions of high light and nutrient availability by increasing the proportion of high-flow tolerant life-forms (Pacheco et al., 2022). As species differ in their tolerance and susceptibility to disturbance, pulsed flows can alter the relative abundances of periphyton taxa (Flinders & Hart, 2009), as well as the relative abundance of disturbance-tolerant versus disturbance-sensitive species (Wu et al., 2019).

Precipitation events are expected to increase in magnitude and frequency in many regions as the climate changes (Huntington, 2006; IPCC, 2014) and in combination with land-use changes that can decrease soil permeability (Aguiar et al., 2015; Aznarez et al., 2021; Zhang et al., 2019), the frequency and magnitude of sudden high flow events will probably increase in many streams. Among those, small lowland streams primarily fed by surface water are likely to be particularly susceptible to sudden high flow events or flow pulses, particularly when situated in agricultural areas with extensive tile draingage (Schilling et al., 2012). Here we define flow pulses or pulse disturbances as sudden, temporarily constrained changes in the environment (Hillebrand & Kunze, 2020; Jentsch & White, 2019), that may induce changes in the species distributional patterns and dynamics (Stanley et al., 2010; Winemiller et al., 2010). Systems subjected to altered flow regimes therefore can undergo rapid ecological changes, and periphyton biomass and composition are considered particularly sensitive variables that can be used as early warnings of changes in flow magnitude and frequency (Guo et al., 2020; Pacheco et al., 2022; Reaver et al., 2019).

Climate change acts on top of other global and local changes, such as the widespread intensification of land use in many catchments worldwide (Sabater et al., 2018) with an expansion of the crop area to the detriment of natural vegetation covers (Bender et al., 2018). Canopy reduction, for instance, leads to a change in irradiance reaching the water, potentially increasing the biomass of primary producers (Julian et al., 2011; Kristensen et al., 2013) that benefit from an increased light supply (McCall et al., 2017; Tank et al., 2018). Even small changes in light availability, resulting from forest clearing, can have profound effects on in-stream primary production (Graça et al., 2018). Moreover, increased use of fertilisers and other agrochemicals inevitably leads to higher phosphorus (P) and nitrogen (N) loads to streams by surface and subsurface runoff, depending on the local current and future climate (Gao et al., 2014; Goyenola et al., 2015, 2020; Jeppesen et al., 2009; Ockenden et al., 2016). This may result in a wide range of environmental, social and economic problems, as diffuse source-induced eutrophication is one of the main causes of the deterioration of aquatic ecosystems across the stream-to-marine continuum (Jabłońska et al., 2020; Kronvang et al., 2007). A recent study by McDowell et al. (2020) estimated that 31% of catchments worldwide already have excessive

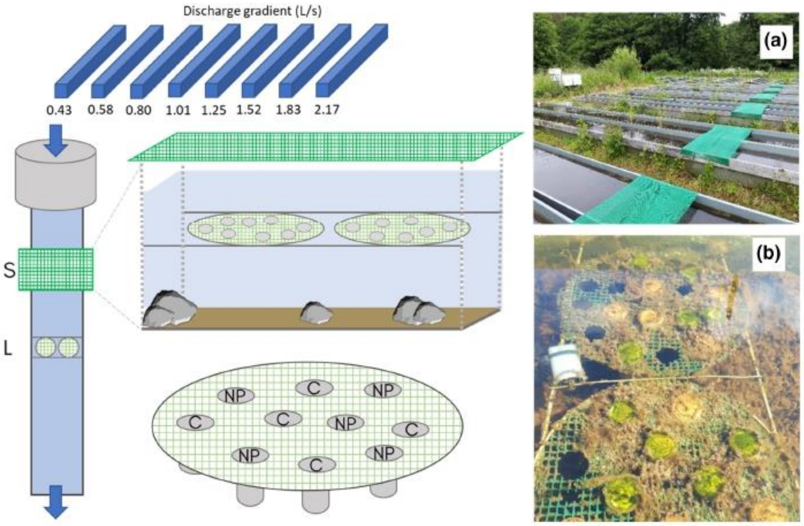

FIGURE 1 Experimental setup including eight outdoor 12-m-long flumes (a) with a continuous baseflow gradient (0.43, 0.58, 0.80, 1.01, 1.25, 1.52, 1.83 and 2.17 L/s). The nutrient diffusing substrates (NDS) for periphyton colonisation, with and without nutrient enrichment (C and NP, respectively), were randomly placed in each flume in a shaded (S) or with total access to natural light (L) sections, as shown. The pictures show the flumes and the supporting devices with the NDS after 4 weeks of colonisation. The empty spots did not have NDS installed (b)

periphyton growth, of which 74% is due to P enrichment and 26% to N enrichment, and that the effects may be stronger when increasing nutrient concentrations co-occur with low-flow conditions (Townsend et al., 2008).

Nutrient and light surplus can lead to high periphyton biomass accrual that may generate both aesthetic and management problems in lotic ecosystems (McDowell et al., 2020). Understanding the effects of temporary abrupt flow pulses, and their interaction with light and nutrient availability on the periphyton community is needed to allow predictions of effects on ecosystem functioning under future scenarios of riparian cover loss, accelerated eutrophication and increased discharge variability that can serve to inform stream management under climate change. To date, several studies have investigated the effects of light, nutrients and flow pulses as single factors or combining two of the parameters as stressors (Adámek et al., 2016; Bækkelie et al., 2017; Bondar-Kunze et al., 2016; Goldenberg-Vilar et al., 2021; Wu et al., 2019), but less is known about the simultaneous effects of the three factors.

Given this background, and based on experience from previous works on independent and interactive effects in streams, we used a multi-stressor design and controlled conditions to better predict the effects of these simultaneous local and global change stressors. We conducted an experiment using artificial streams to test whether flow pulses had different effects on the biomass and composition of periphyton along a gradient of baseflows, under contrasting availabilities of light and nutrients. The gradient in baseflow was chosen as representative for the lower range of small lowland streams in temperate zone (Pedersen et al., 2004). We tested two hypotheses: (1) at lower baseflows a flow pulse would enhance periphyton growth via enhanced transfer of nutrients (i.e., nutrient replenishment), whereas at higher baseflows, a flow pulse would reduce the biomass of the periphyton due to increased scouring. These contrasting mechanisms acting at low and high flow would likely lead to a unimodal response of biomass to flow pulses. (2) High nutrient and light availability would enhance periphyton growth and drive compositional changes through species turnover, probably compensating for the potential negative effects of flow pulses at the high end of baseflows.

2 | METHODS

2.1 | Experimental design

We used a gradient of eight baseflows (0.43, 0.58, 0.80, 1.01, 1.25, 1.52, 1.83 and 2.17 L/s) including two contrasting conditions of light (Shade vs. Light) and two levels of nutrients (substrates with N + P enrichment vs. Control without nutrient addition) combined in four different conditions (Treatments: Shade Control, Light Control, Shade NP and Light NP). Each of these different conditions was replicated five times on each stream.

The experiment was conducted in eight outdoor flumes at the Lemming experimental facility belonging to Aarhus University, Jutland, Denmark during summer 2019 (Figure 1). The flumes were rectangular (12 m long, 60 cm wide, 30 cm deep) channels that were fed from 1,000-L plastic tanks with unfiltered water pumped from the nearby Lemming stream (56°14′N, 9°31′E) with a continuous unidirectional flow and no recirculation. The baseflow in each flume was regulated by flow controllers installed at the pipes before the respective feeding tanks. The artificial streams had a 5% slope and their bottoms were covered with fine sediment, sand, gravel and randomly located rocks. More specific details about the flumes can be found in Neif et al. (2017). At the beginning of June 2019, a pre-experimental phase started when the eight flumes were adjusted to generate a continuous baseflow gradient ranging from 0.43 to 2.17 L/s, allowing the drift and colonisation of periphyton throughout the flumes for a month.

The open flumes were exposed to natural light conditions, with a 16 hr:8 hr, light:dark photoperiod; thus, high light conditions typical for the northern summer. By placing a green plastic mesh (size 1 × 1 mm) approximately 10 cm above the water in some parts of the flumes, we created two conditions of light intensity (Light and Shade), simulating the shade created naturally by the riparian vegetation canopy. The average photosynthetically active radiation (PAR) at the surface level was, near 12:00 hr, 823 ± 451 μmol m^{-2} s^{-1} in the light treatment and significantly lower in the shade treatment (reduction of 50%, average 427 ± 286 μmol m^{-2} s^{-1}) in both surface and subsurface water (Figure 2). Manipulative experiments have tested a wide range of PAR reductions, with the most common being c. 90%, as canopies can reduce the incident PAR by >95% (Winkelmann et al., 2014), and with the minimum value usually about 50% or 60% (e.g., Hill et al., 2011; Kiffney et al., 2004; Mosisch et al., 1999; Quinn et al., 1997).

Using nutrient diffusing substrates (hereafter NDS; Trochine et al., 2014), we generated two concentrations of nutrients: Control (without nutrient addition) and NP (enriched) = 0.5 M NH_4NO_3 + 0.03 M KH_2PO_4, to simulate a scenario of eutrophication in the latter. The amount of nutrients in the NDS were defined according to a balanced ratio for algal growth of 16:1 (molar) N:P ratio (Redfield, 1958; Reynolds, 2006), simulating a balanced eutrophication scenario to avoid limitation by a particular nutrient. Both treatments had equal access to the basal nutrients in the water column. NDS consisted of 30 ml acrylic vials filled with agar-agar solution (with or without added nutrients) and after the mixture had solidified, we placed GF/F fibreglass filters (25 mm diameter) on top of each vial covering the agar, and held these filters with holed lids, exposing a 2-cm-diameter circle on the top. The exposed area of the filters served as a substratum for periphyton growth. Before starting the experiment, we performed a short assay to analyse the nutrient release capacity from the enriched NDS by placing them and one control without nutrients in a known volume of distilled water and dark conditions, and measured nutrient concentrations for 16 days. The diffusion from substrates showed a continuous linear trend (more details in Pacheco

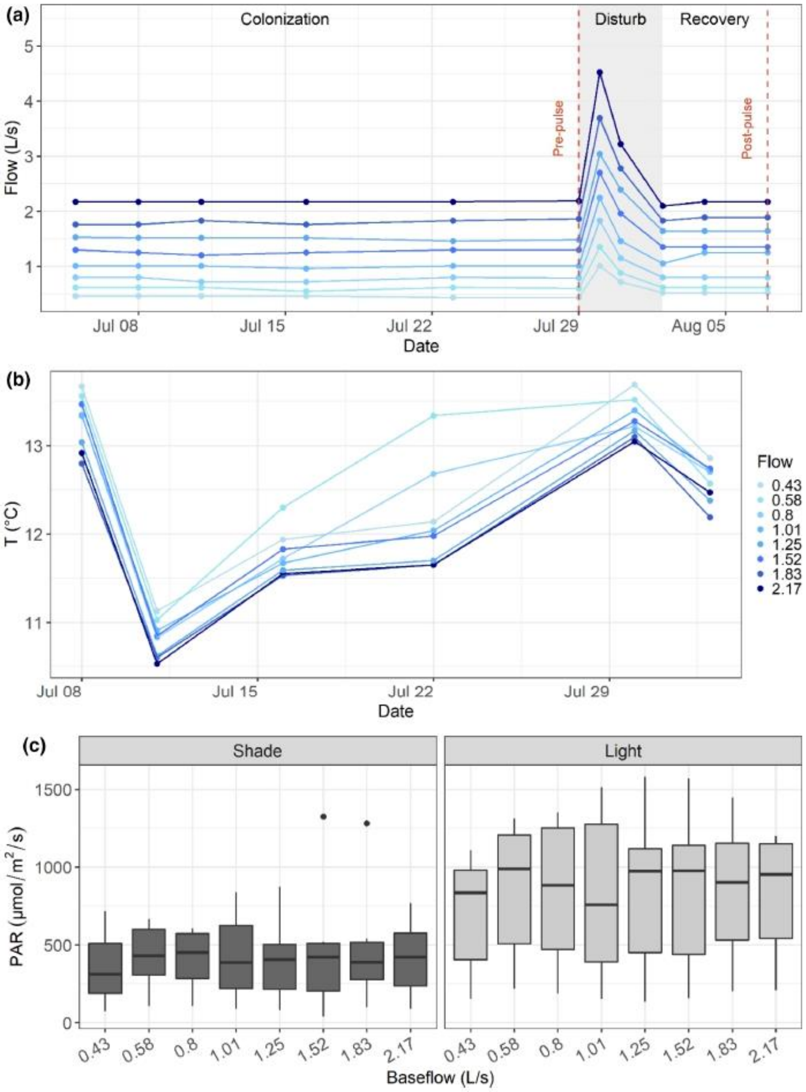

FIGURE 2 (a) Flows (L/s) recorded during the experiment in the eight flumes. The colonisation period was from 1 July to 29 July (pre-pulse sampling), followed by the disturbance period when the flow was increased to the double and then taken back to baseflow values for 7 days (post-pulse sampling). (b) Temperature (°C) recorded in the water of the flumes during the experiment. (c) Mean photosynthetically active radiation (PAR) values reaching the substrates during the experiment in the Shade and Light treatments et al., 2022). The localised effect on the growth of the periphyton on the filter was ensured by the low nutrient diffusion resulting from the small size of the NDS and the low nutrient concentrations used (as shown in similar designs in Pacheco et al., 2022; Trochine et al., 2014). This design restricts the nutrient diffusion, thus maintaining treatments with and without nutrients fairly independent on one another, even in standing waters, as had been found in previous experiments (Trochine et al., 2014).

After a month with the pre-set baseflows, we started the pre-pulse colonisation phase. On 1 July, we randomly assigned the NDS (with and without the addition of nutrients) to each of the flumes and within the flumes to 50% light reduction (Shade) or with no coverage, fully open to light (Light). The substrates were placed on supporting structures and submerged 5 cm below the surface, allowing for periphyton colonisation by natural drift from the stream during four weeks.

After the colonisation phase, we removed half of the NDSs of the four treatments (Shade Control, Light Control, Shade NP and Light NP) from the flumes (pre-pulse sampling) and transported the periphyton-colonised substrates in cold and dark boxes to the laboratory. This phase lasted 4 weeks, a time considered suitable for periphyton succession on artificial substrata (Szabo et al., 2008) while avoiding NDS nutrient depletion. The resulting communities at the end of the colonisation period were analysed in detail (see Pacheco et al., 2022). Then, the flumes containing the remaining substrata were exposed to an abrupt increase in the discharge, as a potential disturbance, for one day (two-fold peak during 24 hr, range 1.01–4.50 L/s), and then a reduction in discharge on the subsequent two days (1.5-fold during 48 hr) and on Day 4 a further reduction until the initial baseflow values were resumed (Figure 2). This increase was expected to act as a disturbance of sufficient magnitude to produce a response in the periphyton assemblage (*sensu* Glasby & Underwood, 1996). The increased discharge in all flumes was proportional to the baseflows so that the periphyton from each flume was exposed to the same relative impact. On Day 8, after four days with basal baseflows, the remaining half of the substrates were removed from the flumes (post-pulse sampling; Figure 2) and transported to the laboratory in cold and dark boxes.

Water temperature and light in the subsurface of each flume were measured every 30 min with a HOBO logger (Hobo Pendant UA-002, Onset). Oxygen concentration, conductivity, pH and turbidity, as NTU, were measured every week (Oxyguard probe and YSI 650 MDS multiprobe). Water samples for total (TP and TN) and dissolved nutrients determination (PO_4-P, NO_3-N and NH_4-N) also were collected weekly from the water column of each flume. Nutrient concentrations were determined following the Danish/European standards for total nitrogen (TN, Danish standard 221, 1975), total phosphorus (TP, Danish standard 292, 1985), and Danish Standard/EN ISO 10304-1 (1996) for orthophosphate (PO_4-P), ammonia (NH_4-N) and nitrate (NO_3-N). Light at 5 cm depth was measured weekly with a LI-COR plane quantum PAR sensor, under both Light and Shade conditions.

2.2 | Periphyton analysis

In this study, we exclusively considered the primary producer component of the periphyton (hereafter periphyton), including both attached or associated organisms (Azim et al., 2005; Wetzel, 1983). The colonised filters from the artificial substrates were carefully divided into two halves for analysis of chlorophyll *a* and taxonomic composition. Periphyton chlorophyll *a* was extracted with 96% cold ethanol in the dark for 24 hr. After centrifugation, chlorophyll *a* and phaeophytin contents were determined spectrophotometrically (Shimadzu 1800 UV/Vis spectrophotometer) before and after acidification with 0.1 mM HCl to correct by phaeophytin content (Jespersen & Christoffersen, 1987; Lorenzen, 1967; Nusch, 1980).

The content on the other half of the filters was preserved in 1% acidified Lugol´s iodine solution for identification and counting. The composition of the periphyton assemblages was analysed under the microscope (Leitz Labovert FS) in random fields at ×400 where ≥100 individuals of the dominant genera in the samples were counted (Lund et al., 1958). Organisms were identified at ×400 to ×1,000 magnification to the lowest taxonomic level possible following Bellinger and Sigee (2015), Biggs and Kilroy (2000) and Wehr et al. (2015). We calculated the biovolumes of each taxon by measuring the length, width and depth dimensions of 10–15 randomly selected organisms following Hillebrand et al. (1999). The organisms were categorised according to type and life forms into the following groups: Chlorophyta (unicellular, colony or filamentous), Cyanobacteria (colony or filamentous), Bacillariophyta (unicellular, colony or filamentous) and others (unicellular algae and filamentous algae from other phyla).

2.3 | Data analysis

A nested design was used because of the hierarchical arrangement of factors given the lack of replicates of baseflow levels. We analysed the periphyton biomass responses to the experimental flow pulse, by comparing the biomass before and after the pulse in the eight different baseflow conditions and under contrasting conditions of light and nutrients with a nested ANOVA, to test for the effects on chlorophyll *a*, richness and biovolume. Significant differences among treatments were identified using Tukey's *post hoc* test.

For the compositional analyses, NMDS ordinations were performed (Oksanen et al., 2017). For this, we used the *metaMDS* function in the VEGAN package in R, and the Bray–Curtis index was used for dissimilarity measures to determine whether the pulse, baseflow, light and/or nutrients led to changes in community structure. We then applied a permutational multivariate analysis of variance (PERMANOVA; Anderson & Walsh, 2013) to test for the significance of the compositional differences between treatments), implemented with the *adonis2* function in R/VEGAN, to compare the composition of samples based on the above factors. SIMPER analysis was used to calculate the contribution of each genus (%) to the dissimilarity between groups of samples (Clarke, 1993).

Ecological resistance, understood as the ability to withstand a perturbation, was calculated according to Hillebrand et al. (2018) using the formula $a = \ln(F_{\text{disturbed}}/F_{\text{control}})$ for biovolume under the whole gradient of baseflows. Benchmark: $a = 0$ = maximum resistance if disturbed = control, $a < 0$ = low resistance through underperformance, $a > 0$ = low resistance through overperformance. We then applied an ANOVA to test the potential effects of light and nutrients, along the flow gradient, on biomass community resistance.

Before each ANOVA test, normality and homogeneity of variance were checked (Shapiro's and Levene's tests, respectively, $p < 0.05$). The statistical analyses were performed using the open-source statistical software package R 3.6.2 (R Core Team, 2019).

3 | RESULTS

3.1 | Flow dynamics, environmental variables and nutrients

During the colonisation period, the only environmental variable that differed among flumes was temperature, which declined with increasing baseflow. The ranges recorded were: temperature 10.5–13.7°C (Figure 2), 9.6–12.2 mg/L O_2, conductivity 322–335 µS/cm, pH 7–7.13 and NTU 2.3–2.5. Dissolved nutrients in water did not differ among baseflow treatments, with average concentrations of 20.9 ± 4.9 µg/L for PO_4, 942 ± 140 µg/L for NO_3 and 6.7 ± 8.5 µg/L for NH_4, and showed low variability during the experiment. Baseflow velocity in the flumes ranged from 0.6 to 3.0 cm/s, and during the pulse it range from 1.4 to 6.3 cm/s.

3.2 | Biomass development and composition

Overall, we found that light availability significantly enhanced periphyton growth, whereas nutrient enrichment only did when combined with full access to light, both before and after the flow pulses (Figure 3; Table 1). Flow also stimulated periphyton growth before the flow pulse but only when combined with full access to light (Figure 3). Following the flow pulse, we found contrasting responses in periphyton growth depending on baseflow conditions. At the lowest baseflows, the pulse promoted periphyton growth, while at higher flows there was a loss of biomass after the pulse (Figure 3), which led to a significant interaction between baseflow and pulse (Tables 1, S1). The linear regressions between baseflows and chlorophyll *a* were evidence for a significant decrease in the pre-pulse scenario for the treatments Shade Control ($R^2 = 0.15$, $p = 0.01$) and a significant increase for Light NP ($R^2 = 0.11$, $p = 0.04$). By contrast, in the post-pulse communities, we found that the higher the baseflow and, thus, the proportional pulse, the lower the remaining periphyton biomass, independent of treatment conditions (Figure 3). The regressions in the post-pulse communities were significant for Shade Control ($R^2 = 0.33$, $p < 0.001$), Shade NP ($R^2 = 0.25$, $p = 0.001$) and Light NP ($R^2 = 0.13$, $p = 0.02$). The maximum positive effect of the pulse on biomass growth occurred in the periphyton exposed to the lowest baseflow with enrichment of nutrients, and full access to light (Figure 3).

Taxonomic richness was higher after the pulse for all baseflows, except for the lowest flow conditions (Figure 4; Table 1), implying also that the interaction effect between pulse and baseflow on taxonomic richness was significant. Nutrient and light availability did not have a significant effect on periphyton taxonomic richness.

Altogether, 68 genera were identified in the samples (27 Chlorophyta, 24 Bacillariophyta, 7 Cyanobacteria, 10 from other groups). The NMDS showed that periphyton composition, in terms of biovolume of each genera found, changed with the pulse disturbance and also differed between the control and nutrient enrichment treatment (Figure 5). Accordingly, the PERMANOVA test revealed pronounced differences in the composition of the pre- and post-pulse communities and among nutrient levels, and to a lesser between the extent of light levels and the baseflow gradient (Table 2). The factor "pulse" accounted for most of the variance ($R^2 = 0.27$), while the factor "nutrients" was the second most important ($R^2 = 0.21$; Table 2). A smaller part of the variance was explained by light level and the baseflow gradient and interactions between nutrient and pulse along the baseflow gradient.

Periphyton biovolume was in most cases higher before than after the flow pulse (Figure 6). The enriched substrates had a higher percentage of chlorophytes, while the nutrient-controls had a higher percentage of diatoms. The composition also was affected by the pulse, mainly resulting in a reduced chlorophytes:diatoms ratio in all treatments. In the nutrient-control treatments, grouping by baseflow and light conditions, the percentage of chlorophytes was <30% and the diatoms constituted >70% before the pulse and changed to <3% and 95% after the pulse, respectively. In the NP treatments, the percentages before the pulse were c. 60% for chlorophytes and <40% for diatoms, changing to 25% and 70% afterwards. The biovolume of cyanobacteria was low in all treatments in both the pre- and post-pulse communities (Figure 6). In the most favourable scenario for periphyton growth (i.e., full access to light and high nutrients), the total biomass increased with increasing baseflow, mainly through enhanced chlorophyte growth, whereas the opposite pattern emerged after the pulse (Figure 6).

The SIMPER analysis revealed that most of the pre- and post-pulse compositional differences reflected a decrease in the biovolume of certain groups after the pulse (Table 3). Thus, the most conspicuous changes from the pre- to post-pulse were a reduction in the biovolume of the diatoms *Encyonema minutum* Hilse and *Ulnaria ulna* Nitzsch and the filamentous Chlorophyta *Oedogonium* spp. in the treatments without nutrients, both under Shade and Light treatments. In the treatments with nutrient addition, the major changes from the pre- to the post-pulse communities were: (a) a large reduction in the biovolume of *Scenedesmus obtusus* Meyen (Chlorophyta) and *Fragilaria capucina* Desmazières, *E. minutum*, *Melosira varians* Agardh and *U. ulna* (Bacillariophyta) under

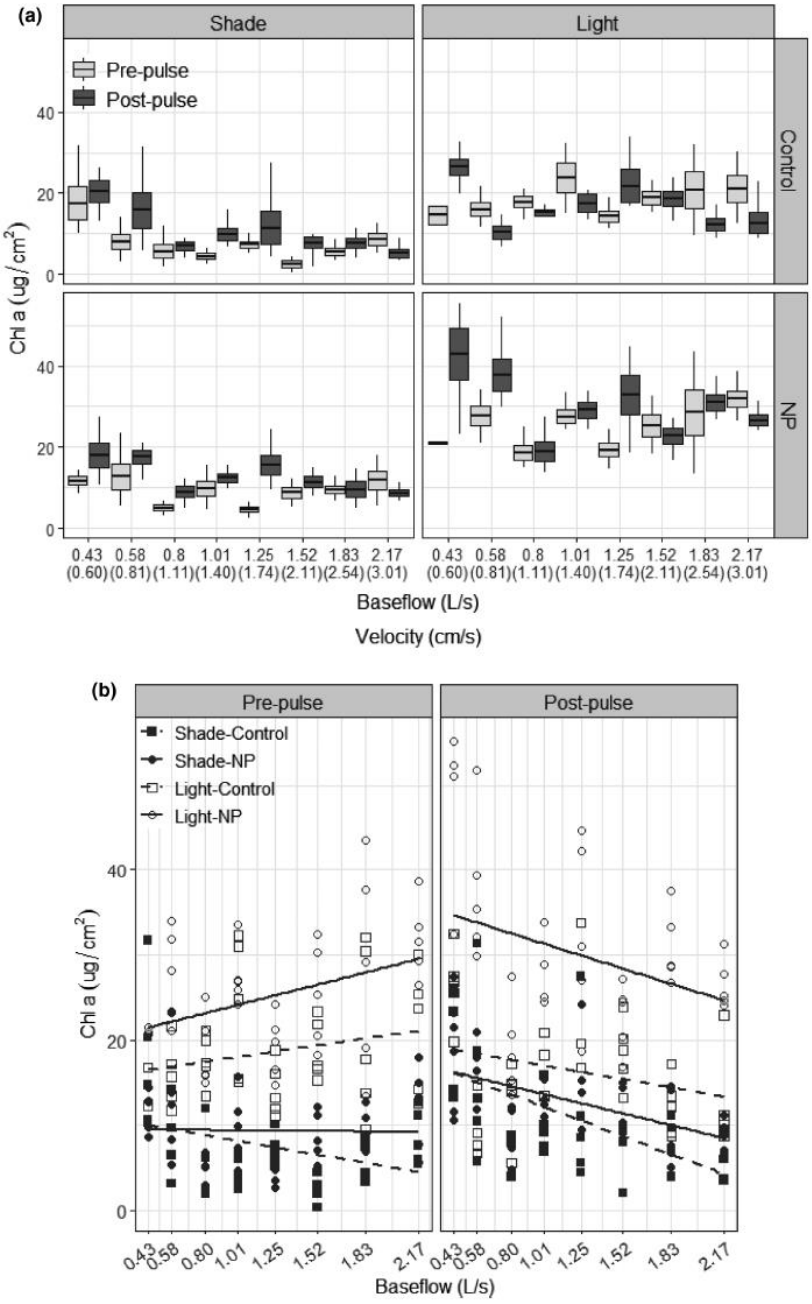

FIGURE 3 (a) Periphyton chlorophyll *a* (μg/cm^2), as a proxy of biomass, showing pre- and post-pulse effects along the baseflow gradient (L/s) for the four combinations of nutrients and light availability (Control Shade, Control Light, NP Shade, NP Light). Mean values, *SEs* (boxes), and minimum and maximum values (whiskers) are shown. Velocity (cm/s) values are shown in parentheses. (b) Linear regressions of chlorophyll *a* with baseflow pre- and post-pulse in the four treatments. Symbols: squares, control (no nutrient addition); circles, NP (nutrient enrichment). Filled shapes correspond to samples from the shaded treatments and the empty shapes to the ones with full access to light. Dashed and solid lines represent treatments without (control) and with (NP) nutrient addition, respectively

shaded conditions, and (b) an increase in *Acutodesmus pectinatus* Meyen (Chlorophyta) and the diatom *F. capucina*, but a decrease in *S. obtusus*, *Chlamydomonas* spp. (Chlorophyta) and *U. ulna* under light conditions.

The periphyton community showed low resistance against the pulse (indicated by underperformance, $a < 0$) regardless of the initial baseflow (although the resistance declined with increasing flow) (Figure 7), and access to full light was associated with higher resistance. Contrarily, nutrients did not have a significant effect on the community resistance against pulse disturbance.

4 | DISCUSSION

In our experiment we demonstrated that the effects of pulses on periphyton have non-linear effects in terms of biomass, as expected, but also that the effects can vary strongly regarding composition depending on the initial community structure, which is in turn mediated by the basal flow, and on light and nutrient availability. The flow pulse promoted a higher taxonomic richness, suggesting a partial reset of the succession of the periphyton communities. Independent of flow and resources, periphyton communities showed low

TABLE 1 Nested ANOVA results on the main effects of experimental factors: pulse (pre- and post-levels), light (Shade and Light, nested in baseflow) and nutrients (Control and NP, nested in baseflow), along the baseflow gradient, and their interactions, on periphyton biomass (chlorophyll *a*) (μg/cm^2, $n = 309$), richness ($n = 128$) and biovolume (mm^3/cm^2, $n = 128$)

Response variable	Factor	*df*	*F*	*p*[a]	*Post-hoc*
Chlorophyll *a*	Pulse	1	18.03	***	See interaction
	Baseflow	7	9.58	***	See interaction
	Pulse*Baseflow	7	7.16	***	Lower range: post- > pre- Higher range: pre- > post-
	Light/Baseflow	8	59.22	***	Light > Shade
	Nutrient/Baseflow	8	13.41	***	NP > Shade
	(Light*Nutrient)/Baseflow	8	4.64	***	Positive
Richness	Pulse	1	70.35	***	Post > pre-
	Pulse*Baseflow	7	2.39	*	Lowest baseflow ≠
Biovolume	Pulse	1	169.36	***	Pre- > post-
	Light/Baseflow	8	3.04	*	Light > Shade
	Nutrient/Baseflow	8	2.90	*	NP > Control

Note: Only significant results for single and multiple stressors are presented (*p*-value < 0.05). Significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

[a] *p*-value.

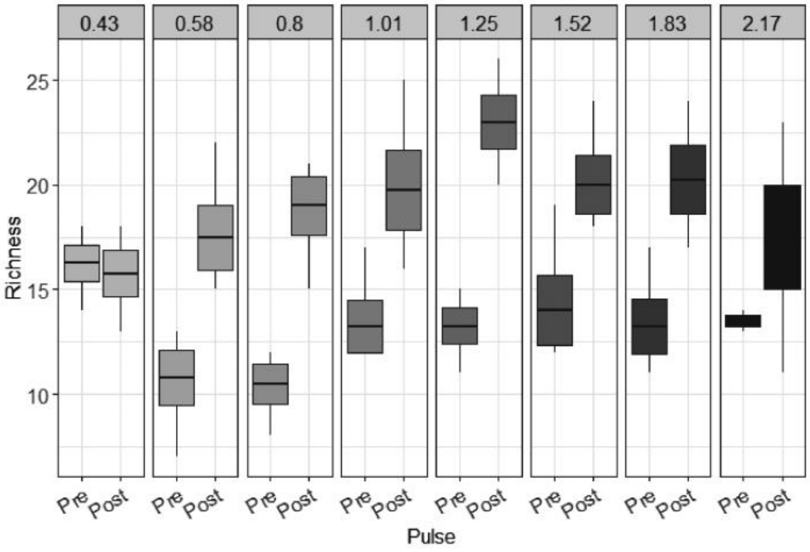

FIGURE 4 Richness before and after the flow pulse along the whole gradient of baseflow (L/s). The color gradient represents the baseflow gradient (L/s). All baseflow replicates are grouped regardless of light and nutrient conditions due to non-significant results. Mean values, *SE*s (boxes), and minimum and maximum values (whiskers) are shown

ecological resistance against the pulse with changes in chlorophyll *a*, biovolume, and taxonomic richness to the pulse.

As predicted (Hypothesis 1), periphyton biomass was constrained both below and above certain values of discharge, expectedly due to nutrient limitation at low and cell removal at high water velocities, respectively (Bækkelie et al., 2017; Hondzo & Wang, 2002; Horner et al., 1990). During pre-pulse, increasing baseflow was beneficial for periphyton biomass accrual only when light was fully available and peaked when combined with nutrient enrichment, as evidenced by the higher biomasses in the combined treatments. Increased flow may enhance the diffusion of solutes to periphyton and thus increase the availability of limiting resources for growth (Huang et al., 2018). However, the comparison between the shaded treatments with and without the addition of nutrients revealed no significant differences, indicating that factors other than nutrients limited the biomass values at low baseflow. Grazers usually have a strong impact on periphyton biomass (Hillebrand, 2009; Moulton et al., 2015; Rosemond et al., 2000), but in our study no clear macroinvertebrate effect on periphyton biomass was observed during the colonisation phase (Pacheco et al., 2022), probably because macroinvertebrates were not fully established in the flumes and thus presented extremely low abundances. We further found that at low basal flow and under unlimited access to light and nutrients conditions, the short-term flow pulse enhanced periphyton growth. This is particularly relevant as lower flow regimes will occur in many places worldwide as a consequence of changes in precipitation patterns. Reduced baseflows are particularly expected if systems are used for water abstraction for drinking purposes and/ or irrigation in agricultural areas (Rolls & Bond, 2017). In areas where longer low-flow periods are expected, changes in magnitude or frequency of flow peaks may occur, with

FIGURE 5 Non-metric multidimensional scaling (NMDS) with Bray–Curtis dissimilarity index illustrating the separation of samples based on the relative abundances of periphyton taxa. Symbols: squares, control/pre-pulse; circles, control/post-pulse; triangles, NP/pre-pulse; diamond, NP/post-pulse. Fill shapes correspond to samples from shaded treatments and empty shapes to the ones with full access to light. The size gradient of the symbols represents the baseflow gradient (L/s). NMDS stress = 0.19. The flow pulse was the major factor driving the abundance of periphyton communities

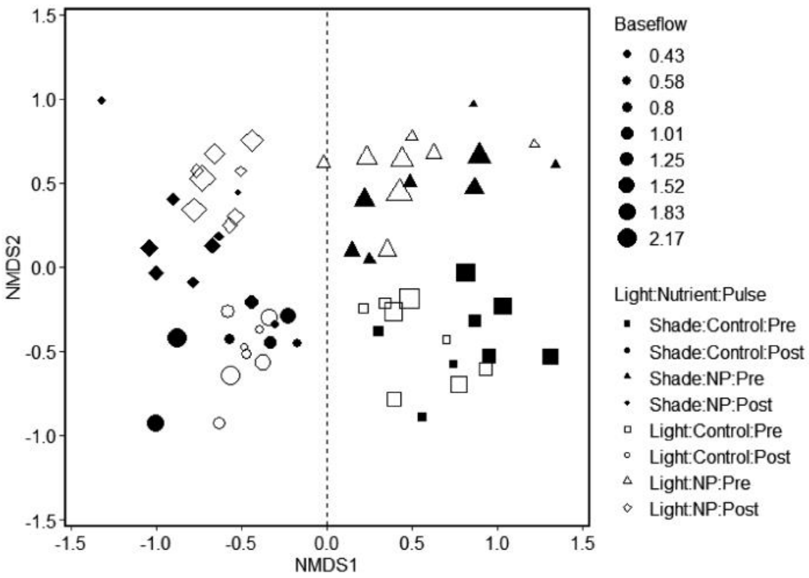

TABLE 2 Nonparametric four-way PERMANOVA on Bray–Curtis distances for the composition of genera of the periphytic algal communities

	df	R^2	*F*	*p*
Pulse	1	0.27	48.63	***^{a}
Baseflow	7	0.09	2.37	***^{a}
Nutrient	1	0.21	37.71	***^{a}
Light	1	0.03	6.16	***^{a}
Baseflow * Pulse	7	0.07	1.83	**^{a}
Nutrient * Pulse	1	0.06	10.43	***^{a}
Nutrient * Baseflow				ns
Nutrient * Baseflow * Pulse				ns

Note: Significance: ***, $p < 0.001$; **, $p < 0.01$.

^{a}*p*-values <0.05.

abrupt and sudden effects on periphyton (Sabater et al., 2018). In our short-term experiment, periphyton reached undesirable levels (12–20 μg/cm^2 according to McDowell et al., 2020) in the treatments with full light availability and low baseflows, and far exceeded the range after the pulse when both light and nutrients were fully available. In the shaded treatments, undesirable chlorophyll *a* concentration was reached only at the lowest baseflows. A consequently higher accrual of periphyton potentially could affect the ecological status of streams (McDowell et al., 2020).

At the higher end of the flow gradient, scouring was probably the prevalent periphyton-removal process during the pulse (Haddadchi et al., 2020). Hoyle et al. (2017) suggested that sand and fine gravel abrasion is the dominant physical mechanism keeping periphyton abundance low in streams during frequent small floods. The potential effect of scouring is strongly influenced by the dominant life forms, which ultimately are conditioned by the environment (Mulholland et al., 1991). In our study, we did observe a reduction in biomass indicating that certain flow threshold values existed. Changes in the regimes of environmental drivers at a regional scale are expected (Poff, 2002). In areas where more frequent extreme rainfall events are predicted, flow pulses will affect stream communities already subject to nutrient enrichment and potentially other impacts.

The second hypothesis, predicting higher growth with nutrient and light availability, which could compensate for potential negative effects of flow peaks (at least at the high end of the baseflow range), was partially supported. Although the magnitude of flow pulses promoted either removal or promotion of periphyton, light and nutrient availability had some local influence on the periphyton biomass. The periphyton biomass in the non-shaded half of the artificial streams in both the pre- and post-pulse phases was significantly higher. This resembles natural systems where the presence of riparian vegetation results in increased shading and, consequently, lower periphytic development (Adámek et al., 2016). As found here, shade can play a more important role in dampening eutrophication effects in streams than nutrients (Schiller et al., 2007), with increasing relevance in a context of climate change.

Effects of nutrients on biomass were not evident but may have been partially masked by the moderately high background levels of nutrients in the flumes, the pronounced effects of disturbance or by the fact that with higher periphytic biomass, there is a decreasing probability of nutrient limitation (Hillebrand, 2002). This could reflect the ability of the periphyton to recycle nutrients internally, leading to lower reliance on external sources so that the positive effects of enrichment decrease with time (Hillebrand, 2002; Mulholland et al., 1994). Nutrients become more important at low removal frequency (Hoyle et al., 2017), and stable flow (Biggs, 1995) and no peak conditions (Bondar-Kunze et al. (2016). Also, Neif et al. (2017) recorded only minor responses of periphytic biomass to nutrient enrichment in an outdoor stream flume experiment using the same facilities. Nutrient enrichment may exert strong effects

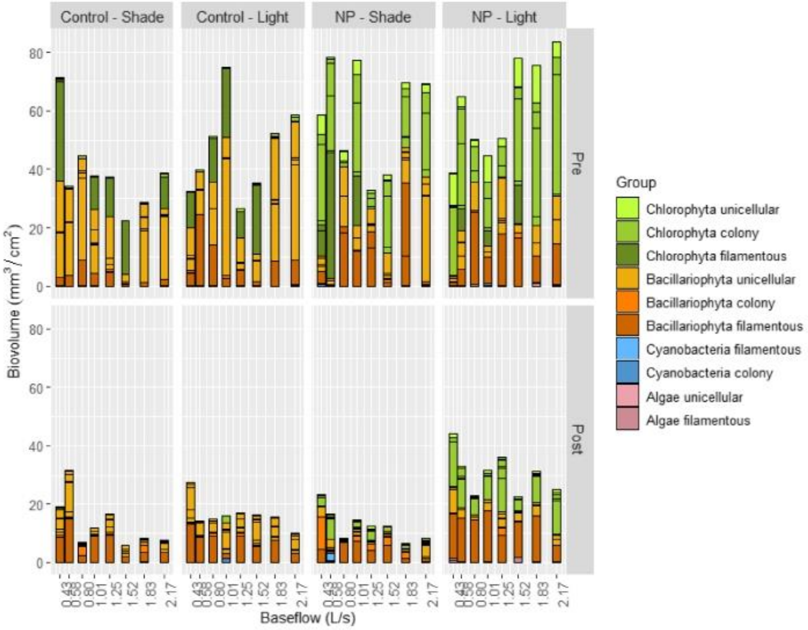

FIGURE 6 Biovolume (mm^3/cm^2) of major periphyton groups present in the experiment pre- (upper panel) and post-pulse (bottom panel) in the four treatments combining conditions of nutrients and light. The lines within the bars denote the different genera found

TABLE 3 List of the genera contributing the most to the different community structures between the pre- and post-pulse as shown by similarity of percentage analysis (SIMPER)

Treatment	Genera	Change	Cumulative %
Control Shade	*Encyonema minutum*	Pre- > post-	31.49
	Oedogonium spp.	Pre- > post-	58.19
	Ulnaria ulna	Pre- > post-	74.38
Control Light	*Oedogonium* spp.	Pre- > post-	26.70
	Encyonema minutum	Pre- > post-	51.41
	Ulnaria ulna	Pre- > post-	71.23
NP Shade	*Scenedesmus obtusus*	Pre- > post-	28.78
	Fragilaria cappucina	Pre- > post-	41.09
	Encyonema minutum	Pre- > post-	52.08
	Melosira varians	Pre- > post-	60.97
	Ulnaria ulna	Pre- > post	69.01
NP Light	*Scenedesmus obtusus*	Pre- > post	30.66
	Acutodemsus pectinatus	Pre- < post-	42.21
	Fragilaria cappucina	Pre- < post-	52.65
	Chlamydomonas spp.	Pre- > post-	62.09
	Ulnaria ulna	Pre- > post-	68.36

Note: The cumulative percentage accounts for the difference between communities relative to treatment (Control Shade, Control Light, NP Shade and NP Light).

when nutrients are limiting and can even have a positive interaction with the increased availability of light (Pacheco et al., 2022; Santos et al., 2018). This also was found in a previous experiment by Hill and collaborators, where they found simultaneous light and P limitation, with light explaining a major fraction of the variance in growth and final biomass of periphyton, and P having a secondary role (Hill & Fanta, 2008; Hill et al., 2009).

For composition (in biovolume), both pulse and nutrient availability had a relevant role as evidenced by differences in the relative abundance of the main groups, while light had subtle effects. Although the experimental flow pulse had a quantitative influence (i.e., a decrease of biomass of loosely attached colonies and filaments), nutrient enrichment had a qualitative effect in determining the community composition (diatom-dominated vs. green algae-dominated), with an effect that prevailed after the pulse. In agreement with our results, Bækkelie et al. (2017) simulated a three-day discharge pulse (0.77 L/s) and found a decrease in chlorophyte and cyanobacterial biomass, but no effects on diatoms. In our experiment, after the flow pulse there were no filamentous chlorophytes left in any of the treatments. This algal group develops only after

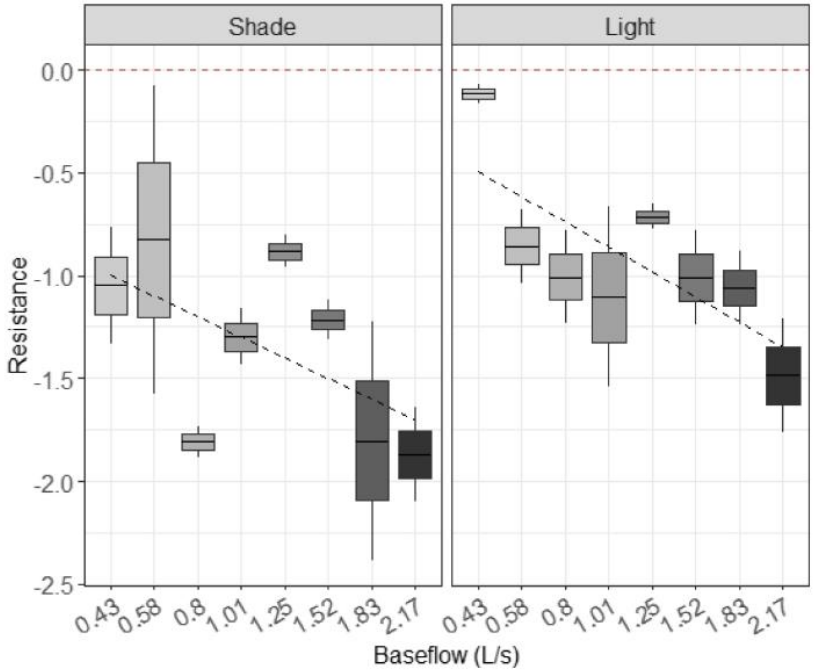

FIGURE 7 Periphyton resistance (*sensu* Hillebrand et al., 2018) against the flow pulse for total biovolume along the original baseflow gradient and the two conditions of light availability (left panel: Shade, right panel: Light). The replicates are grouped irrespective of the nutrient treatment due to non-significant results. Mean values, *SE*s (boxes), and minimum and maximum values (whiskers) are shown

extended periods of low flow (Biggs, 1995). Pérez-Calpe et al. (2021) found an inverse relationship between chlorophyll *a* and flow, even though they tested a low range of water velocities. They proposed that this pattern resulted from dominance of the community by filaments (prone to sloughing) that were lost with increasing flow, which concurs with the findings of other studies (Baattrup-Pedersen et al., 2020; Biggs et al., 1998; Neif et al., 2017; Passy, 2007; Wu et al., 2019). The generalised lower post-pulse biovolumes found, which did not coincide with Chlorophyll *a*, are probably the result of biovolume being a direct measure that involves counting, whereas biomass estimation using Chlorophyll *a* concentration may largely vary depending on the groups, the individual organisms, and also by the position in the periphytic matrix. This generalised response of reduction of biovolumes and compositional changes after the flow pulse showed that the peak effectively disturbed the communities (Death, 2010; Glasby & Underwood, 1996). We found that chlorophytes (unicellular organisms or colonies) only remained after the flow pulse in the nutrient-enriched treatments. It has been demonstrated previously that water nutrient loadings partially shape the composition, structure and function of periphyton (Lu et al., 2016), with a potentially strong qualitative effect on the community composition (diatom-dominated vs. green algae-dominated) (Bondar-Kunze et al., 2016). As demonstrated here, previous studies manipulating nutrients and light simultaneously also found stronger community composition effects of nutrient availability than of light (McCall et al., 2017).

The gradient of baseflows, typical of lowland streams (Graeber et al., 2017; Neif et al., 2017), and the different conditions of nutrient and light availability, led to the development of different periphyton communities during the colonisation phase (as described in Pacheco et al., 2022). Periphyton groups have different life forms that entail different attachment abilities (Baattrup-Pedersen et al., 2020; Horner et al., 1990; Neif et al., 2017; Passy, 2007; Wu et al., 2019), implying that flow acts as a strong filter during the colonisation phase. Under full light conditions, periphyton growth increased with increasing discharge on both the nutrient and nutrient-control substrates, but the response was strengthened when the substrates were enriched. Growth enhancement occurred even when radiation values--in both treatments--were higher than previously suggested values of photosynthesis saturation (Bondar-Kunze et al., 2016; Hill et al., 2009), a response largely regulated by the different pigment compositions of periphyton groups and that cannot be generalised for all types of communities (Falkowski & Raven, 2013). Enrichment alone did not enhance periphyton growth, however, suggesting that light played a stronger role in our experimental setup.

Our results highlight the importance of studying the temporal dynamics of multiple stressors (Sabater et al., 2018). The pulse disturbance promoted an increase in taxonomic richness in the periphyton community in most of the experimental flumes. The higher richness after the pulse could suggest a partial reset of the succession of the periphyton communities and new opportunities for colonisers. Flow disturbances favour distinct genera differentially, with high flow disturbance selecting for small cell sizes, low profile, and firmly attached organisms, and low flow conditions favouring big cell sizes with high profile species, filamentous and motile taxa (Wu et al., 2019). The relationship between baseflow and richness was, however, not linear. The flow range tested here was insufficiently wide to allow the determination of potential threshold values beyond which richness is negatively affected, but this is a promising area for research. While richness was enhanced with the pulse, resistance (calculated for periphyton biovolume) was low and decreased with increasing baseflow. The degree of recovery (i.e., the degree to which a pre-disturbed state is reached) is higher if resistance is high, whereas communities with low resistance usually have high resilience (i.e., the rate of approaching a non-disturbed control) (Hillebrand & Kunze, 2020). Opportunistic species are crucial both during re-colonisation, when surface patches become available (in streams typically after the abrupt input of runoff water), and during the rapid growth phase after the recolonisation (Biggs et al., 1998; Horner et al., 1990). Here, we did not assess resilience, but other experiments have found strong responses of the periphytic community and high resilience to the stress imposed by low flow (Baattrup-Pedersen et al., 2020; Neif et al., 2017). Light availability significantly increased community resistance, which again supports its role as a leading driver of biomass accrual and suggests that local limiting conditions can influence resistance patterns. In a scenario of increasing light reaching streams as a result of deforestation or loss of riparian vegetation and other changes in land use, our results suggest an enhanced periphyton growth and a major resistance against the control naturally exerted by flow pulses. Our findings could be extrapolated, but with caution as biotic interactions such as grazing and bioturbation were obviously weaker than in many natural systems.

Worldwide, degradation of running water ecosystems is partly due to undesirable levels of periphyton biomass that impair human demands of water for agriculture and urban activities, and recreational use (McDowell et al., 2020), and some studies have suggested that manipulation of flows could potentially reduce nuisance periphyton growth (Flinders & Hart, 2009). Here, we demonstrated that the effects of pulses on periphyton are consistent in terms of biomass along the baseflow gradient, but varied strongly regarding composition depending on their initial structure, which is in turn mediated by the basal flow normally experienced by the systems, and on light and nutrient availability. Local environmental settings will ultimately determine the net effects of these extreme events on periphyton community structure and probably also on several important ecosystem processes.

AUTHOR CONTRIBUTIONS

Conceptualisation: CC, MM. Developing methods: JPP, CC, ABP, MM. Conducting the research: CC, JPP, CA. Data analysis: CC, JPP, ABP. Data interpretation: CC, JPP, CA, EJ, ABP, MM. Preparation of figures and tables: CC, JPP. Writing: CC, JPP, CA, EJ, ABP, MM.

ACKNOWLEDGMENTS

We thank Anne Mette Poulsen for editing the manuscript. We are grateful to Carolina Trochine for her valuable support with nutrient diffusing substrata, Yang Liu for her field and laboratory assistance, and Ann Lene Vigh for conducting the nutrient analyses. We also thank the editor and the reviewers for their contributions that helped to improve the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

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SUPPORTING INFORMATION

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How to cite this article: Calvo, C., Pacheco, J. P., Aznarez, C., Jeppesen, E., Baattrup-Pedersen, A., & Meerhoff, M. (2022). Flow pulses shape periphyton differently according to local light and nutrient conditions in experimental lowland streams. *Freshwater Biology*, *00*, 1–15. https://doi.org/10.1111/fwb.13916

Supplementary material

Table S1. Nested ANOVA results on the main effects of experimental factors: pulse (pre and post levels), baseflow (gradient of 8 levels), light (shade and light, nested in flow) and nutrients (control and NP, nested in flow), and their interactions, on periphyton biomass (chlorophyll *a*) (n=309), richness (n=128), and biovolume (n=128). Only significant results for single and multiple stressors are presented (p-value<0.05).

Response variable	**Factor**	**Sum sq**	**Mean sq**	**Df**	**F**	**p**
Chlorophyll *a*	Pulse	8.37	8.37	1	18.03	***
	Baseflow	31.11	4.45	7	9.58	***
	Pulse*Baseflow	23.27	3.32	7	7.16	***
	Light/Baseflow	219.91	27.49	8	59.22	***
	Nutrient/Baseflow	49.78	6.22	8	13.41	***
	(Light*Nutrient)/Baseflow	17.24	2.16	8	4.64	***
Richness	Pulse	576.0	576.0	1	70.35	***
	Baseflow	92.7	13.2	7	1.64	ns
	Pulse*Baseflow	136.8	19.5	7	2.39	*
	Light/Baseflow	70.3	8.8	8	1.09	ns
	Nutrient/Baseflow	55.8	7.0	8	0.87	ns
	(Light*Nutrient)/Baseflow	68.7	8.6	8	1.07	ns
Biovolume	Pulse	137.56	137.56	1	169.36	***
	Baseflow	9.39	1.34	7	1.65	ns
	Pulse*Baseflow	13.01	1.86	7	2.29	ns
	Light/Baseflow	19.72	2.47	8	3.04	*
	Nutrient/Baseflow	18.85	2.36	8	2.90	*
	(Light*Nutrient)/Baseflow	5.47	0.68	8	0.84	ns