UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CENTRO DE AQUICULTURA DA UNESP

# Produção de tambaqui *Colossoma macropomum* em cultivo multitrófico integrado com curimba *Prochilodus lineatus*

**Adriana Ferreira Lima**

Jaboticabal, São Paulo

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**Orientador: Dr. Wagner C. Valenti**

Tese apresentada ao Programa de Pós-graduação em Aquicultura do Centro de Aquicultura da UNESP - CAUNESP, como parte dos requisitos para obtenção do título de Doutor.

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# SUMÁRIO

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## Resumo

Esta tese comparou a produção de tambaqui (*Colossoma macropomum*) em monocultivo e em sistema de cultivo multitrófico integrado (IMTA) com curimba (*Prochilodus lineatus*). Para isso, foram conduzidos quatro estudos complementares que avaliaram: (i) os efeitos da inclusão da curimba no desempenho produtivo, saúde dos peixes, qualidade da água e viabilidade econômica; (ii) as interações tróficas entre as espécies e o uso de recursos alimentares naturais; (iii) os impactos ambientais do sistema integrado por meio de Avaliação do Ciclo de Vida; e (iv) os fluxos difusivos e ebulitivos de gases de efeito estufa. A inclusão da curimba não comprometeu o crescimento, a sobrevivência, a produtividade ou a taxa de conversão alimentar do tambaqui, aumentando em 25% a biomassa total de peixes e reduzindo em 14% a conversão alimentar, indicando maior eficiência no uso da ração. A bioturbação promovida pela curimba alterou principalmente parâmetros físicos da água, favorecendo a liberação de nutrientes e estimulando cascatas tróficas do tipo *bottom-up*. Nesse contexto, a curimba atuou como espécie extrativa, consumindo zooplâncton e contribuindo para a redução da matéria orgânica. O IMTA aumentou a eficiência ecotrófica da ração e do zooplâncton. O sistema integrado elevou a receita líquida em US$ 1.481,14 ha ano$^{-1}$ e reduziu impactos ambientais, como eutrofização de água doce e dependência hídrica. A integração da curimba não resultou em aumentos nos fluxos de $CH_4$ e $N_2O$. Esses resultados indicam que o IMTA de tambaqui e curimba aumenta a eficiência produtiva e representa uma alternativa mais sustentável aos monocultivos convencionais.



**PALAVRAS-CHAVE**

Análise de ciclo de vida, espécies de baixo nível trófico, Ecopath, gases de efeito estufa, IMTA, isótopos estáveis, policultivo

## Abstract

This thesis compares the production of tambaqui (*Colossoma macropomum*) in monoculture and in integrated multitrophic aquaculture (IMTA) systems with curimba (*Prochilodus lineatus*). Four complementary studies were conducted to evaluate: (i) the effects of curimba inclusion on fish performance, health, water quality, and economic viability; (ii) trophic interactions between the species and the use of natural food resources; (iii) the environmental impacts of the integrated system through Life Cycle Assessment (LCA); and (iv) diffusive and ebullitive greenhouse gas fluxes. The inclusion of curimba did not compromise tambaqui growth, survival, productivity, or feed conversion ratio, increasing total fish biomass by 25% and reducing feed conversion by 14%, indicating greater feed-use efficiency. Bioturbation promoted by curimba mainly altered physical water parameters, favoring nutrient release and stimulating bottom-up trophic cascades. In this context, curimba acted as an extractive species, consuming zooplankton and contributing to the reduction of organic matter. IMTA increased the ecotrophic efficiency of feed and zooplankton. The integrated system increased net revenue by US$ 1,481.14 $ha^{-1}$ $year^{-1}$ and reduced environmental impacts such as freshwater eutrophication and water dependence. The integration of curimba did not result in increased $CH_4$ and $N_2O$ fluxes. These results indicate that tambaqui–curimba IMTA improves production efficiency and represents a more sustainable alternative to conventional monoculture systems.



**KEY WORDS**

Ecopath, greenhouse gases, IMTA, Life Cycle Assessment, low trophic level species, polyculture, stable isotopes

## Introdução geral

A comercialização de produtos de origem aquática atingiu valores equivalentes ao total movimentado pelo comércio de carnes terrestres em 2022, evidenciando a relevância do pescado no mercado alimentar global (Boyd et al. 2020; Checa et al. 2024; FAO 2025). A demanda por pescado vem aumentando continuamente, impulsionada pelo crescimento da população mundial e pela busca por alimentos nutritivos (FAO 2025).

A aquicultura aparece como uma solução viável para atender a essa demanda, contribuindo para o fortalecimento da segurança alimentar e da nutrição em escala global, no presente e para as próximas gerações (Boyd et al. 2020; Checa et al. 2024; FAO 2025). Embora os países asiáticos se destaquem como grandes produtores aquícolas por meio do policultivo e de sistemas integrados, como a rizipiscicultura, o monocultivo intensivo segue em expansão e permanece amplamente adotado em escala global (Thomas et al. 2021). No entanto, o monocultivo apresenta limitações quanto ao uso eficiente dos recursos, podendo resultar em uma descarga elevada de nutrientes no ambiente (Lecocq et al. 2024). Portanto, é fundamental reavaliar o monocultivo como modelo para a expansão da aquicultura mundial, de modo a promover seu crescimento sustentável e garantir alinhamento com os objetivos da Agenda 2030 para o Desenvolvimento Sustentável (FAO 2025). Na aquicultura, a sustentabilidade depende da adoção de práticas e tecnologias que aumentem a produtividade e reduzam os impactos ambientais (Boyd et al. 2020). Alcançar esse equilíbrio requer o uso eficiente de recursos, como energia, água, solo, ração e fertilizantes. A intensificação da aquicultura por meio de sistemas integrados multitróficos tem sido proposta como uma estratégia para aumentar a sustentabilidade do setor, ao melhorar a utilização de recursos e a recuperação de nutrientes na forma de biomassa animal (Rahman et al. 2006; Chopin et al. 2012; Boyd et al. 2020; Lecocq et al. 2024; Chary et al. 2025). Nesse estudo, adotamos a definição de cultivo integrado proposta por Boyd et al. (2020), na qual espécies que ocupam diferentes nichos tróficos são co-cultivadas em proporções adequadas, a fim de otimizar o uso de recursos e reduzir a emissão de

poluentes ambientais. Esse conceito está inserido no contexto mais amplo do policultivo, que consiste no cultivo simultâneo de múltiplas espécies com fins produtivos (Lecocq et al. 2024). Dentro desse escopo, a Aquicultura Multitrófica Integrada (IMTA) pode ser definida como uma forma especializada de policultivo, na qual espécies que ocupam diferentes nichos tróficos e funcionais são co-cultivadas de modo que os produtos metabólicos (por exemplo, ração não consumida, excretas e nutrientes dissolvidos) de um componente sejam utilizados como insumos por outro, promovendo maior eficiência no uso de recursos, reciclagem de nutrientes e redução dos impactos ambientais (Boyd et al., 2020).

Um dos principais desafios do cultivo integrado multitrófico é selecionar espécies compatíveis para o co-cultivo, de modo a evitar interações negativas, ao mesmo tempo em que estas sejam complementares na utilização dos recursos. Nessa seleção, as espécies devem ocupar diferentes níveis tróficos para maximizar o aproveitamento dos recursos disponíveis. Entre os sistemas integrados, o sistema mais estudado é o policultivo de carpas, praticado na China desde os séculos VII a IX (Milstein 1992). No entanto, as carpas não são candidatas ideais para a aquicultura ocidental, devido à sua baixa aceitação pelo mercado consumidor e ao seu baixo valor de mercado (Thomas et al. 2021). No Brasil, o policultivo de carpas vem sendo praticado nas últimas quatro décadas, mas atualmente representa uma contribuição limitada para a produção aquícola nacional, já que seu consumo é predominantemente regional (Valenti et al. 2021). Portanto, para promover o cultivo integrado multitrófico como estratégia de aumento da sustentabilidade da aquicultura, é necessário identificar espécies alternativas que complementem os monocultivos tradicionais e sejam atraentes para sua adoção pelo setor. Com base nisso, propomos um sistema de cultivo integrado combinando tambaqui (*Colossoma macropomum*) e curimba (*Prochilodus lineatus*).

O tambaqui é a espécie nativa mais cultivada no Brasil, com produção de aproximadamente 160 mil toneladas em 2024 (IBGE, 2026), concentrada principalmente na região Norte, mas também presente nas regiões do Centro e Sul do país (Hilsdorf et al. 2022). É uma espécie pelágica com capacidade de consumir

alimentos naturais, tanto em seu habitat natural (Goulding and Carvalho 1982; Silva et al. 2000) quanto em ambientes de cultivo, ao longo de todos os estágios de vida (Sipaúba-Tavares and Braga 2007; Gomes and Silva 2009a; Lima et al. 2024a, b). Peixes do gênero *Prochilodus* (conhecidos no Brasil como curimba, curimatã, curimatá ou curimbatá, e como bocachito ou sábalo em outros países da América do Sul) apresentam produção aquícola no Brasil em torno de 3,3 mil toneladas por ano (IBGE, 2026). São detritívoros migratórios, de baixo nível trófico, e estão entre as espécies de água doce mais abundantes e amplamente distribuídas nos rios da América do Sul (Sivasundar et al. 2001). Essas características os tornam candidatos ideais para sistemas integrados multitróficos, pois permitem recuperar nutrientes depositados nos sedimentos. O cultivo de curimba é tradicional no Brasil e ocupa a quinta posição entre as espécies nativas mais produzidas para consumo humano (Valenti et al. 2021). A integração dessas espécies está alinhada ao conceito de compatibilidade intrínseca discutido por Thomas et al. (2021), que aponta o cultivo integrado de espécies bentônicas e pelágicas como forma de reduzir a competição interespecífica. Essa combinação de espécies também se enquadra na definição de aquicultura integrada de nível 1 proposta por Boyd et al. (2020b), que classificam os níveis de integração com base nas interações ecológicas entre as espécies co-cultivadas. Nesse sistema, as espécies são produzidas no mesmo ambiente, mas ocupam nichos ecológicos distintos, ou seja, dependem de recursos diferentes ou desempenham funções complementares dentro do sistema.

Alguns estudos já investigaram o co-cultivo de curimba e tambaqui. Hancz (1993), por exemplo, publicou o primeiro estudo avaliando o co-cultivo de tambaqui com carpa capim (*Ctenopharyngodon idella*) e curimba (*Prochilodus marggravii*). Este estudo demonstrou a viabilidade do co-cultivo dessas espécies, embora não tenha comparado os resultados do policultivo com os do monocultivo. Franchini et al. (2020) avaliaram a integração de tambaqui com curimba (*Prochilodus lineatus*) e camarão amazônico (*Macrobrachium amazonicum*) durante a fase juvenil. Eles observaram que o co-cultivo não interferiu no crescimento do tambaqui, mas melhorou tanto o rendimento total das espécies quanto a taxa de conversão

alimentar. Esses estudos evidenciam o potencial de produzir tambaqui em sistemas integrados multitróficos sem comprometer o seu crescimento. No entanto, eles foram conduzidos apenas durante a fase inicial de produção. Considerando a elevada biomassa de peixes e os elevados custos com ração nos estágios finais do período de engorda, torna-se essencial avaliar a viabilidade do cultivo integrado de tambaqui durante toda essa fase do ciclo de produção.

Os benefícios da integração de espécies em sistemas aquícolas não devem ser avaliados apenas por indicadores convencionais da aquicultura, como desempenho de crescimento, custos de produção, qualidade da água e saúde dos peixes. É igualmente necessário investigar os mecanismos ecológicos envolvidos na integração, incluindo o uso de recursos alimentares naturais e as interações tróficas entre as espécies. Essas interações influenciam a eficiência no uso de recursos e a produtividade do sistema (Granada et al. 2018). A análise de isótopos estáveis é um método que permite quantificar a contribuição de diferentes fontes alimentares para o crescimento dos peixes, especialmente em sistemas nos quais as espécies podem competir pelos mesmos recursos. A modelagem de ecossistemas aquáticos complementa essa abordagem, ao avaliar a dinâmica das espécies dentro de um contexto ecológico mais amplo, permitindo otimizar combinações de espécies com base na produtividade e na biodiversidade do viveiro (Heymans et al. 2004; Aubin et al. 2021). Isto é particularmente relevante em sistemas integrados multitróficos, nos quais as espécies cultivadas interagem com comunidades biológicas intrínsecas ao ecossistema, como fitoplâncton, zooplâncton e macroinvertebrados bentônicos (Thomas et al. 2021).

Além disso, quantificar o desempenho ambiental dos sistemas aquícolas é fundamental para identificar abordagens realmente sustentáveis. A Avaliação do Ciclo de Vida (ACV) tem se destacado como uma ferramenta relevante nesse contexto (Ghamkhar et al. 2021), pois permite integrar diferentes categorias de impacto ambiental ao longo da cadeia produtiva. Entretanto, a robustez das análises de ACV depende fortemente da qualidade e da representatividade dos dados utilizados, especialmente no que se refere às emissões diretas de gases de efeito

estufa (GEE) nos sistemas de cultivo, que ainda são frequentemente estimadas (Aubin et al. 2009, 2015; Jerbi et al. 2012; Mungkung et al. 2013). Entre esses gases, o metano e o óxido nitroso merecem atenção especial devido aos seus elevados potenciais de aquecimento global (IPCC, 2021), sendo o $CH_4$ frequentemente apontado como o principal contribuinte das emissões aquícolas (Yuan et al. 2019). O $N_2O$, apesar de normalmente apresentar contribuição quantitativamente menor, deve ser considerado para uma avaliação integrada e abrangente das emissões do setor. Apesar de o $CO_2$ ser classificado como gás de efeito estufa, suas emissões em sistemas aquícolas são tratadas como biogênicas, pois representam o retorno de carbono recentemente assimilado pelos organismos (Stichnothe and Hospido 2008; Hischier et al. 2010; Biermann and Geist 2019), razão pela qual não são usualmente contabilizadas nos inventários de impacto climático. Assim, a quantificação integrada desses gases é indispensável tanto para a acurácia dos estudos de ACV quanto para o entendimento dos ciclos biogeoquímicos responsáveis pela geração e transformação dos gases de efeito estufa em sistemas aquícolas.

Estudos comparativos de ACV entre monocultivos e sistemas integrados ainda são limitados e os resultados disponíveis frequentemente apresentam inconsistências. Enquanto alguns trabalhos relatam benefícios ambientais do policultivo ou da Aquicultura Integrada Multitrófica (IMTA), como a redução dos impactos sobre as mudanças climáticas e a eutrofização (p.ex., Cao et al. 2013; Pouil et al. 2024), outros indicam melhorias insignificantes, dependendo da escolha das espécies e da configuração do sistema (p.ex., Medeiros et al. 2017). Ainda assim, sistemas integrados geralmente apresentam desempenho superior ao monocultivo quando este envolve espécies de menor produtividade ou maiores taxas de conversão alimentar (Hala et al. 2024). Apesar da importância do tambaqui na aquicultura brasileira, os benefícios ambientais e econômicos de sua integração com outras espécies, como o curimba, permanecem inexplorados.

Atualmente, observa-se uma crescente demanda pelo poder público por modelos de produção mais sustentáveis na aquicultura brasileira, especialmente na

região amazônica, onde o tambaqui é a espécie predominante e os impactos ambientais do monocultivo convencional são significativos. Nesse contexto, este estudo busca preencher essa lacuna de conhecimento, avaliando a integração tambaqui-curimba por meio de indicadores de desempenho e saúde dos peixes, da utilização de alimentos naturais e das interações tróficas entre as espécies, assim como dos aspectos ambientais e econômicos desse modelo de produção. Tem-se como objetivo fornecer informações sobre o potencial dos sistemas integrados multitróficos aplicados à produção de tambaqui para aumentar a eficiência no uso de recursos, reduzir impactos ambientais e promover o desenvolvimento sustentável da aquicultura no Brasil.

Esta tese está organizada em quatro artigos. Em três deles, a análise baseia-se na comparação entre o monocultivo de tambaqui e o sistema de cultivo multitrófico integrado (IMTA) com curimba, ambos com tambaqui em densidade comercial. No segundo artigo, além desses tratamentos, foi incluído um tratamento adicional de cultivo integrado com menor densidade de estocagem do tambaqui, a fim de avaliar como diferentes proporções entre as espécies influenciam as interações tróficas e o uso de recursos alimentares naturais. O primeiro artigo foca na avaliação do impacto da inclusão da curimba na produção de tambaqui, considerando o desempenho e a saúde dos peixes, parâmetros de qualidade da água e aspectos econômicos. O segundo artigo examina as interações tróficas entre as espécies integradas e o uso de recursos alimentares naturais, incluindo a influência das proporções de cada espécie nessas interações e na utilização dos recursos. O terceiro artigo avalia o impacto ambiental do sistema integrado por meio de análise de ACV. O quarto artigo avalia, ao longo do ciclo produtivo, os fluxos difusivos e ebulitivos de gases de efeito estufa na produção de tambaqui em monocultivo e em sistema integrado com curimba.

## Manuscrito 1

Este manuscrito foi submetido para o *Journal of World Aquaculture Society* e retornado para correções pelos autores.

# Integrating curimba culture during the grow-out phase of tambaqui affects overall productivity, water quality parameters and fish health

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## Author Contribution Statement

A.F. Lima and W.C. Valenti conceived and designed the study. A.F. Lima and A.S. Pereira planned and conducted pond experiments, collected all samples, and participated in data analysis. L.N. Ganeco-Kirschnik and P.O. Maciel-Honda assisted with sample collection and contributed to data analysis. A.F. Lima drafted the manuscript with input and revisions from all co-authors. All authors approved the final version of the manuscript and agreed to its submission for publication.

## Abstract

Integrated multi-trophic aquaculture (IMTA) improves nutrient efficiency by integrating extractive species with fed species. This study assessed the impact of including curimba (*Prochilodus lineatus*) in tambaqui (*Colossoma macropomum*) production during the grow-out phase in ponds. Two systems were compared: tambaqui monoculture and tambaqui with curimba IMTA, each evaluated in triplicate. The addition of curimba did not affect tambaqui growth, feed conversion ratio, or survival but increased total fish yield by approximately 25%. Besides, it led to lower morning dissolved oxygen and afternoon pH, and higher levels of chlorophyll, turbidity, total dissolved and suspended solids in the water. Phytoplankton densities were unaffected, probably because they were controlled by the zooplankton, which increased in integrated systems. Monogenean parasite abundance in tambaqui was significantly higher in the integrated system. Implementing the integrated culture, increased net profit by US$ 1481.14 per hectare per year. Further research should explore different tambaqui and curimba ratios to optimize curimba growth and increase total productivity. This study indicates that tambaqui can be effectively farmed in IMTA systems, together with benthic species, enhancing production efficiency and supporting circular economy principles.


**Key-words:** *Colossoma macropomum,* economic analysis, integrated multitrophic aquaculture, monogenea, polyculture, *Prochilodus.*

## Introduction

The total net trade of aquatic animal products was comparable to the total value of trade in all terrestrial meats in 2022 (FAO 2025). The growing global population and increasing demand for healthy food are driving a steady rise in the demand for fish protein. Aquatic food systems present a viable solution, offering significant opportunities to enhance global food security and nutrition now and for future generations (Boyd et al. 2020; Checa et al. 2024; FAO 2024). In 2022, aquaculture surpassed capture fisheries in aquatic animal production for the first time, accounting for 51% of the world's total fish production (FAO 2024). However, this growth has negatively impacted on the environment, causing reduced land availability, water eutrophication and other forms of pollution, toxic chemical accumulation, and threats to the food chain (Ahmad et al. 2021). Thus, changes in aquatic food systems are necessary to achieve sustainable aquaculture growth and contribute to the 2030 Agenda for Sustainable Development (FAO 2024).

In aquaculture, achieving environmental sustainability involves selecting practices and technologies that enhance productivity while minimizing environmental impact (Boyd et al. 2020). To mitigate the negative effects on the environment, extensively studied ecological approaches promote aquaculture production designs that reduce nutrient discharges (Checa et al. 2024). Sustainability in aquaculture hinges on the efficient use of resources, such as energy, water, land, feed, and fertilizer. Reducing the use of these resources is key to providing healthy food for a growing population in an environmentally responsible manner (Boyd et al. 2020). Fed monoculture forms the foundation of global aquaculture, but production that relies on a single species is often inefficient in its use of natural resources (FAO 2024). In general, only a tiny proportion of the nutrients from the feed supplied in aquaculture systems are assimilated by fish, and the unutilized nutrients are retained in the pond sediments or released in the effluents (Sahu et al. 2015; David et al. 2017a, b; Flickinger et al. 2019, 2020b, a; Barbosa et al. 2024).

The Integrated multi-trophic aquaculture (IMTA) is a promising solution to increase nutrient efficiency in aquaculture (Troell et al. 2009). Polyculture refers to

a farming system in which two or more species are cultivated simultaneously, typically combining species that utilize different ecological niches or natural resources (Troell et al. 2009; Lecocq et al. 2024). Integrated Multi-Trophic Aquaculture (IMTA) builds upon this concept by cultivating fed species alongside extractive species, such as filter feeders and nutrient-absorbing plants or algae, in appropriate proportions. This approach creates balanced systems that promote environmental and economic sustainability (Boyd et al. 2020). In IMTA systems, nutrients released by one cultured species are utilized by other species at different trophic levels, enhancing resource use efficiency and protein production. This approach distributes feed costs across multiple commercial crops, captures carbon, prevents nutrient loss, and maximizes meat protein yield from plant-based ingredients systems (Troell et al., 2009; Chopin et al. 2012; Boyd et al. 2020). The concept of IMTA is traditionally applied to marine aquaculture, where fish production is integrated with filter-feeding organisms, such as mussels and oysters, along with seaweed species that utilize dissolved nutrients. However, in freshwater pond aquaculture, which constitutes the majority of global aquaculture, there is no established technology for using filter-feeding organisms and seaweed species. Therefore, selecting suitable extractive species to complement those currently raised in fed monoculture is crucial for enhancing the sustainability of aquaculture production.

Fish of the genus *Prochilodus* are large, migratory and detritivore that occupy low-trophic level and ranked among the most prominent, abundant, and widely distributed freshwater species in South American rivers (Sivasundar et al. 2001). This makes them an ideal candidate for IMTA, as they recover nutrients settled in the sediment from primary species production in pond systems. Farming curimba (*Prochilodus spp.*) is traditional in Brazil and constitutes the fifth most produced group of native species for human consumption (Valenti et al. 2021, IBGE 2026). Considering the potential of this species for use in an IMTA system, we evaluated its integration with tambaqui *Colossoma macropomum*, the most widely farmed native species in South America (Hilsdorf et al. 2022). Tambaqui exhibits a remarkable ability to consume natural food in natural habitats (Goulding

and Carvalho 1982; Silva et al. 2000) and in rearing ponds during all life stages (Sipaúba-Tavares and Braga 2007; Gomes and Silva 2009a; Lima et al. 2024a, b). As curimba is a detritivorous demersal fish that eats dead organic matter (Kalous et al., 2012), it has a complementary food habit in relation to tambaqui, which is a crucial characteristic for selecting secondary species in IMTA systems (Lecocq et al. 2024).

Some studies have explored the inclusion of curimba in tambaqui production together with a third species. No research focusing on only tambaqui and curimba was found in the literature. Hancz (1993) evaluated the polyculture of tambaqui with curimba (*Prochilodus marggravii*) and grass carp (*Ctenopharyngodon idella*) over a 164-day period. This study demonstrated the feasibility of co-cultivating these three species together, although it did not compare the outcomes with those from monoculture. Franchini et al. (2020) evaluated the integration of tambaqui with curimba (*Prochilodus lineatus*) and Amazon River prawn (*Macrobrachium amazonicum*) during juvenile production (53 days). They found no negative impact on tambaqui performance, and the integration improved both the overall yield of all species and the feed conversion ratio. These studies highlight the potential to produce tambaqui in integrated systems without compromising growth. However, they were conducted during the initial production phase. It is essential to evaluate the feasibility of integrated culture during the entire grow-out phase. In the later stages, the fish biomass, feed supplied, and oxygen consumption are high, and a second species might affect the water and tambaqui performance. Therefore, the present study aims to evaluate the impact of including curimba in tambaqui production during the grow-out phase in ponds, focusing on fish performance, water quality, plankton community, fish health, and economic aspects.

## Materials and Methods

### *2.1 Experimental design and fish culture*

The study was conducted at the Aquaculture Experimental Center of Brazilian Agricultural Research Corporation in Palmas, Tocantins, Brazil

(10°8'1.33"S, 48°19'9.86"W). A completely randomized experiment was designed with two treatments and three replicates per treatment. The factor tested was the type of production system with two levels: (T) tambaqui *Colossoma macropomum* monoculture (0.4 fish $m^{-2}$) and (TC) tambaqui (0.4 fish $m^{-2}$) with curimba *Prochilodus lineatus* (0.3 fish $m^{-2}$) in an integrated multitrophic aquaculture system. The tambaqui stocking density corresponded to the common practice of the commercial grow-out phase of this species (Valenti et al. 2021). Curimba stocking density was adapted from previous experiments with this species in IMTA (e.g., Franchini et al., 2020), considering the proportion between the two species. The experiment duration was planned to obtain the commercial size (1.7 kg) of the target species, tambaqui. Thus, the experiment lasted seven months (210 days). Sampling times were defined as T0 to T7, where T0 corresponded to the beginning of the experiment (fish stocking) and the subsequent times represented the months of cultivation. A batch of tambaqui fingerlings (2.0 ± 0.8 g) and curimba (3.2 ± 2.1 g) were purchased from a commercial hatchery (Brejinho de Nazaré, TO, Brazil; 48◦35′17,93”S, 11◦1′52,95”W and Ipueiras, TO, Brazil; 48°27’54.274’’S, 10°58’14.88’’O, respectively), maintained in ponds and fed with extruded commercial feed (1 - 2.6 mm pellet size and 45% crude protein) until apparent satiety four times a day until achieve the initial size to this study. Then, six earthen ponds (600 $m^2$, 1.3 m deep) were stocked with 60-day-old tambaqui (*Colossoma macropomum*) (29 ± 4 g). Three of the ponds were randomly selected and also stocked with 60-day-old *curimba* (*Prochilodus lineatus*) (15.5 ± 8.7 g). Prior to the experiment, ponds were drained, disinfected with quicklime (200 g $m^{-2}$), limed (200 g limestock $m^{-2}$), and fertilized (5 g urea $m^{-2}$, 3 g simple superphosphate $m^{-2}$ and 10 g rice bran $m^{-2}$), all previously diluted in water before application to the ponds (Lima et al., 2024a). Subsequently, the ponds were filled with water from a local dam. Throughout the study, ponds received water just for reposition of evaporation and seepage.

The target species was tambaqui, which was fed twice a day (8:00 h and 16:00 h) with commercial extruded feed (Archer Daniels Midland Company-ADM, Brazil). Fish with a weight range of 30 to 230 g, 230 to 410 g, and 410 to 700 g

were fed a diet containing 32% crude protein, at feeding rates of 4.5%, 3%, and 2% of body weight per day, respectively. For fish weighing between 700 and 1,130 g, and those exceeding 1,130 g, a feed containing 28% crude protein was provided at feeding rates of 2% and 1.5% of body weight per day (Oliveira et al., 2013). Each month, 30 tambaqui and 30 curimba were randomly sampled from each pond and weighed to monitor the growth. Then, they returned to their respective ponds. Only tambaqui biomass was considered when adjusting the amount of feed offered. Uneaten feed that floated on the pond surface was negligible, so the feed supplied was used as a proxy for actual feed intake. The apparent feed conversion ratio (FCR) was calculated using the formula: FCR = total feed supplied / total weight gain. At the end of the experiment, ponds were drained, and all fish were harvested and weighed in batches of five fish, and total yield was recorded. Productivity was calculated as the ratio of final biomass to pond area and converted to t $ha^{-1}$ $year^{-1}$, while survival was determined as the percentage of the initial number of fish that survived to the end of the experiment.

*2.2 Water quality parameters*

Water variables were monitored regularly. Temperature, dissolved oxygen, pH, and conductivity were measured three times a week at 08:00 and 16:00 using a YSI Professional Plus probe (Yellow Springs Instruments Company, Yellow Springs, USA). Transparency was recorded three times a week at 08:00 with a Secchi disk. Each pond was sampled monthly at 08:00 for the analysis of total and dissolved phosphorus, chlorophyll-a, total suspended solids, dissolved solids, and turbidity. These parameters were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 2017), using the following procedures: total phosphorus by persulfate digestion method (4500-P B.5), chlorophyll-a by acetone extraction followed by spectrophotometric determination (10200 H), total suspended solids by the gravimetric method (2540 D), dissolved solids by the gravimetric method (2540 C). Total nitrogen was determined by catalytic combustion oxidation using an Elementar Vario TOC-N Select analyzer (Langenselbold, Germany), and turbidity was analyzed with a turbidimeter (Hach

2100Q, Loveland, CO, USA). For statistical analyses, all measurements were averaged monthly, generating one value per pond per month for each variable. Whenever dissolved oxygen levels fell below 3 mg/L, supplemental aeration was applied using fountain-type aerators (1.5 HP), operating from 18:00 to 08:00, 14 hours per day.

*2.3 Plankton availability*

The availability of phyto- and zooplankton in the ponds was analyzed on months 0, 2, 4, 6, and 7, by sampling phyto- and zooplankton using phyto- and zooplankton nets (20 and 68 μm mesh, respectively). The nets were dragged for 10 m in the earthen ponds, with a total volume of 709 L for phytoplankton and 1075 L for zooplankton analysis. Phytoplankton was preserved in 2% formaldehyde neutralized with sodium bicarbonate (5 g $L^{-1}$). The zooplankton samples were preserved in 4% formaldehyde neutralized with sodium bicarbonate (5 g $L^{-1}$) and 0.1% rose Bengal dye. Phyto- and zooplankton samples were quantitatively analyzed using Neubauer and Sedwick Rafter chambers, respectively.

*2.4 Parasitic analysis*

Five tambaqui from each pond were sampled in months 5 and 7. The fish were euthanized by severing the spinal cord, and gill samples were collected and fixed in a 4% buffered formalin solution until analysis. The first branchial arch from the right side of each fish was examined for total monogenean parasite counts using a stereo microscope (Stemi305S, Zeiss, Germany). Prevalence, mean intensity and abundance indexes were calculated according to Bush et al. (1997). For monogenean species identification, 60 parasites from each treatment group were treated in Hoyer’s medium, and microscope slides were prepared to visualize identification structures (haptor and copulatory organ) (Eiras et al. 2006). Monogenean identification followed the methods outlined by Cohen et al. (2013).

*2.5 Partial budget analysis*

Enterprise budgets include all the relevant costs and returns from a particular enterprise. However, as indicated by Engle (2010), a partial budget is

often more suitable for analyzing the impact of small changes in the production process. A partial budget focuses only on the income and expense items that will be affected by the proposed modification. Thus, in the present study, we considered the production costs associated with curimba juveniles and the additional labor required for curimba stocking and harvest. For curimba stocking, an additional labor time of 5.5 hours per hectare was considered. For curimba harvesting, the extra time was estimated proportionally to the biomass harvested, based on the time required to harvest only tambaqui. We assume that harvesting 250 kg of tambaqui (~1 pond of 600 m²) requires 2 hours per worker, with a team of four workers. This time includes 20 minutes for material preparation, 30 minutes for the first dragging of the fishing net, 40 minutes for handling and removing the fish from the net, and an additional 30 minutes for a second net dragging, if necessary. The unit cost of curimba juveniles was US$ 0.04, the selling price of 1 kg of harvested curimba was US$ 1.85, based on prices informed by fish farmers from northern Brazil, and the monthly labor cost for a worker employed 22 days per month, 8 hours per day, was US$ 369.58. Both values were obtained in Palmas, Tocantins, Brazil, in June 2024. All monetary values are presented in U.S. dollars (US$ 1.00 = R$ 5.41, exchange rate from August 2024).

*2.6 Statistical analysis*

Data was subjected to tests of normality (Shapiro-Wilk test) and homogeneity (Bartlett test) of residues and transformed (Box and Cox 1964) when they did not meet the premises. Data for fish mass, survival, yield, and FCR were subjected to Student's *t*-test. While water quality parameters, phytoplankton, zooplankton, rotifers, copepods and cladocerans density and parasites were subjected to repeated measures ANOVA, with treatment, culture period, and their interaction considered as fixed effects in the model. Experimental units were considered as random effects. When significant ANOVA was obtained, the means were compared using the Tukey test. The data were presented as the mean ± standard deviation, and statistical significance was assumed at a level of *P* close to

or lower than 0.05. All statistical analyses were performed using the 4.2.3 R software (R Core Team, 2024).

*2.7 Legal and Ethical Aspects*

The study complied with official Brazilian guidelines for the care and use of animals for scientific and educational purposes (Concea-CEUA protocol 76/2022), and with the National Management System for Genetic Heritage and Associated Traditional Knowledge (AB47B85).

## Results

The inclusion of curimba in the tambaqui ponds did not affect tambaqui growth and survival and improved tambaqui productivity (Table 1). The integrated system increased total yield by approximately 25%. The feed conversion ratio (FCR) of tambaqui remained unaffected by the addition of curimba, while the total FCR of the integrated system decreased about 12%, though no significant difference was observed (Table 1).

TABLE 1 The mean ± standard deviation of the productive performance of tambaqui (*Colossoma macropomum*) monoculture (T) and integrated culture of tambaqui and curimba (*Prochilodus lineatus*) (TC) in ponds

| | Monoculture | IMTA | *P*-value |
|---|---|---|---|
| *Tambaqui* | | | |
| Initial individual mass (g) | 29 ± 4 | 29 ± 4 | NA |
| Final individual mass (g) | 1693 ± 307 | 1776 ± 194 | 0.7136 |
| Survival (%) | 58 ± 14 | 60 ± 14 | 0.8397 |
| Yield (kg/ha/year) | 6485 ± 657 | 7056 ± 943 | 0.4258 |
| FCR tambaqui | 1.73 ± 0.16 | 1.73 ± 0.25 | 0.9851 |
| *Curimba* | | | |

| | | | |
|---|---|---|---|
| Initial individual mass (g) | - | 15 ± 9 | - |
| Final individual mass (g) | - | 144 ± 44 | - |
| Survival (%) | - | 97 ± 6 | - |
| Yield (kg/ha/year) | - | 962 ±307 | - |
| | | | |
| FCR total species | 1.73 ± 0.16 | 1.52 ± 0.23 | 0.2698 |
| Total Yield (kg/ha/year) | **6485 ± 657 b** | **8028 ± 943 a** | 0.0831 |

Notes. FCR – Feed conversion ratio; Different letters in the same row indicate significant differences by *t*-test.

The addition of curimba in the system decreased morning dissolved oxygen and afternoon pH. Additionally, it increased the amount of chlorophyll and nitrogen in the water, as well as turbidity and total dissolved and suspended solids. Dissolved oxygen levels fell below 3 mg/L in some ponds between the fourth and fifth months, requiring the use of supplemental aeration. The average number of days the aerator was used ranged from 0 to 53 days in T and from 0 to 78 days in TC, with no significant difference between treatments ($P$ = 0.9319). Although most water quality parameters varied over time (Table 2), no clear temporal trend was observed. A significant interaction between treatment and culture period was observed only for morning pH, suggesting a synergistic effect. Higher pH values were recorded in the tambaqui monoculture during the second and third months. Phytoplankton and zooplankton densities showed no significant variation during the culture. Phytoplankton density was higher in the integrated system; however, no significant differences were observed between treatments. Rotifer densities were significantly higher in ponds with curimba (Table 3), while cladoceran and copepod densities did not differ significantly between the two culture systems.

TABLE 2 The mean ± standard deviation of the water quality parameters measured in tambaqui (*Colossoma macropomum*) monoculture (T) and integrated culture of tambaqui and curimba (*Prochilodus lineatus*) (TC) in ponds

| | | Treatments | | *P* value | | |
|---|---|---|---|---|---|---|
| | | T | TC | Treatment | Time | Treat*Time |
| Temperature (°C) | Morning | 29 ± 1.8 | 28.9 ± 1.6 | 0.5486 | <0.0001 | 0.682 |
| | Afternoon | 32.2 ± 1.8 | 32.1 ± 1.6 | 0.0614 | <0.0001 | 0.3053 |
| Dissolved oxygen (mg $L^{-1}$) | Morning | **6.0 ± 1.7 a** | **5.5 ± 1.6 b** | 0.0280 | 0.0011 | 0.0890 |
| | Afternoon | 10.0 ± 2.3 | 9.9 ± 2.0 | 0.9255 | 0.1512 | 0.1114 |
| Conductivity (µS $cm^{-1}$) | Morning | 92.4 ± 29.8 | 110.4 ± 24.8 | 0.2482 | 0.0001 | 0.9868 |
| | Afternoon | 103.5 ± 32.1 | 120.2 ± 23.2 | 0.2009 | 0.0006 | 0.9893 |
| pH | Morning | 8.0 ± 0.7 | 7.9 ± 0.7 | 0.2384 | <0.0001 | 0.0312 |
| | Afternoon | **8.8 ± 0.5 a** | **8.6 ± 0.4 b** | 0.0183 | 0.0958 | 0.1065 |
| Total Nitrogen (mg L-1) | Morning | **1.02 ± 0.57 b** | **1.32 ± 0.62 a** | 0.0838 | 0.2162 | 0.2292 |
| Total Phosphorus (µg L-1) | Morning | 83.5 ± 44.5 | 93.5 ± 56.1 | 0.5123 | <0.0001 | 0.3534 |
| Total Dissolved Phosphorus (µg L-1) | Morning | 32.5 ± 19.8 | 32.8 ± 18.3 | 0.8675 | <0.0001 | 0.4562 |
| Chlorophyll-a (mg L-1) | Morning | **19 ± 27.4 b** | **34.5 ± 37.2 a** | 0.0442 | 0.8384 | 0.1592 |
| Total Dissolved Solids (mg L-1) | Morning | **86.6 ± 41 b** | **104.7 ± 55.6 a** | 0.0216 | <0.0001 | 0.4271 |
| Total Suspended Solids (mg L-1) | Morning | **11.8 ± 10.9 b** | **25.6 ± 24.7 a** | 0.0117 | 0.0001 | 0.4417 |
| Transparency (cm) | Morning | **92.3 ± 32.7 a** | **65.8 ± 33.8 b** | 0.0107 | 0.0992 | 0.3547 |
| Turbidity (NTU) | Morning | **28.5 ± 29.3 b** | **49 ± 41.1 a** | 0.0022 | 0.0482 | 0.7848 |

Notes. Treat – Treatment; Different letters in the same row indicate significant differences by ANOVA, followed by the Tukey test.

TABLE 3 The mean ± standard deviation of plankton density measured in tambaqui (*Colossoma macropomum*) monoculture (T) and integrated culture of tambaqui and curimba (*Prochilodus lineatus*) (TC) in ponds

| | Treatments | | *P* value | | |
|---|---|---|---|---|---|
| | T | TC | Treatment | Time | Treat*Time |
| Phytoplankton (individuals L-1) | 2314 ± 2374 | 3203 ± 2854 | 0.2734 | 0.3520 | 0.2376 |
| Zooplankton (individuals L-1) | **430 ± 352 b** | **806 ± 530 a** | 0.0389 | 0.2541 | 0.9370 |
| Rotifer (individuals L-1) | **109 ± 110 b** | **325 ± 374 a** | 0.0325 | 0.5145 | 0.4911 |
| Copepods (individuals L-1) | 319 ± 299 | 409 ± 314 | 0.3004 | 0.0132 | 0.9255 |
| Cladocerans (individuals L-1) | 1.18 ± 2.43 | 1.14 ± 1.99 | 0.9132 | 0.1139 | 0.4536 |

Notes. Treat – Treatment; Different letters in the same row indicate significant differences by Tukey test.

Three species of monogenean parasites were identified in the gills of tambaqui: *Anacanthorus spathulatus, Notozothecium janauachensis,* and *Mymarothecium boegeri* (Monogenoidea). The prevalence, regardless of the species, was 100% in tambaqui in both monoculture and integrated systems at 5 and 7 months. The mean abundance of monogenean parasites was significantly lower in tambaqui monoculture (287 ± 164 individuals) compared to integrated culture (472 ± 148) ($P = 0.0002$). Additionally, the time of evaluation (5 or 7 months) did not significantly affect parasite intensity ($P = 0.5027$).

The inclusion of curimba in the integrated system resulted in the production of approximately 962 kg/ha/year of curimba biomass, leading to an additional revenue of US$ 1,777.42 per hectare per year (Table 4). Production costs also increased by US$ 296.28 per hectare per year, mainly due to the purchase of

curimba juveniles. Overall, the integration of curimba increased net benefits by US$ 1481.14 per hectare per year, representing the difference between the added revenue and costs, and reflecting the financial gain from adopting the integrated system (Table 4).

TABLE 4 Partial budget analysis of curimba (*Prochilodus lineatus*) inclusion in pond-based tambaqui (*Colossoma macropomum*) culture

| Category | Value (US$/ha/year) |
|---|---|
| *Benefits* | |
| Additional revenue | 1777.42 ± 568.58 |
| *Additional costs* | |
| Juvenile | 209.14 |
| Labor (stocking and harvesting) | 87.14 |
| *Net Benefit (Benefits - Additional costs)* | 1481.14 ± 568.58 |

Notes. Price data were obtained in Palmas, Tocantins, Brazil, in June 2024; Monetary values are expressed in U.S. dollars (US$ 1.00 = R$ 5.41, exchange rate accessed in August 2024).

**Discussion**

The inclusion of curimba inside tambaqui ponds did not negatively affect tambaqui growth, survival, productivity and feed conversion ratio. Apparently, no negative interactions between the two species occurred. Adding curimba to the culture increased total fish production without additional feed. Most water quality parameters and plankton communities were influenced by the presence of curimba. The integrated culture increased revenue and profit.

Pond aquaculture experiments frequently have a limited number of replicates per treatment, typically three (e.g. Costa et al., 2025; Dantas et al., 2020; Zhang et al., 2024), because of the shortage of experimental ponds at research stations. This setup means that only substantial differences between treatments will be statistically detectable (Smart et al. 1998). Additionally, significant variability between ponds is typical, as each pond, along with its unique community of flora

and fauna, forms an independent ecosystem that can differ considerably from other experimental ponds, even before treatments are applied. This inherent variability can complicate the identification of clear patterns and trends in the assessed variable, further challenging the detection of treatment effects (Riley and Edwards 1998). In the present experiment, the use of three replicates per treatment resulting in only 6 experimental units, but the variability in the tambaqui performance was relatively low (CV ranged from 11 to 24%). In addition, numerical values were higher in the integrated culture than in monoculture for all variables, and *P*-values ranged from 0.42 to 0.98. Thus, it is reasonable to assume that the performance of the tambaqui was similar in both systems of culture. On the other hand, the high variability in data of phytoplankton and copepod densities associated with low number of trials raises the risk of Type II error, where we may mistakenly conclude that no differences exist between treatments when, in fact, they might. As such, caution must be applied in interpreting the results within the context of these limitations.

The final individual mean mass, survival and total yield of tambaqui in monoculture were comparable to those in integrated culture, indicating that curimba did not compete with tambaqui for space or food. This finding is consistent with observations by Franchini et al. (2020) in tambaqui juvenile production. The final individual mass of tambaqui (about 1700 g) was higher than typically expected for a seven-month culture, as this weight is usually achieved in at least 10 months (Lima et al., 2024a; Lima et al., 2025; Valenti et al., 2021). This accelerated growth may be attributed to the lower survival rate observed in this study (~60%), which decreased the density inside ponds and shortened the culture period by three months. The reduction of at least 30% in the production cycle duration, due to lower stocking densities, offers an opportunity for farmers who prioritize more frequent cycles over maximum yield per cycle, enabling greater financial turnover. No meaningful mortalities were noted in the ponds, and the low survival in both treatments was likely due to cormorant (*Nannopterum brasilianum, former Phalacrocorax brasilianus*) predation observed during the second and third months

of the experiment. Cormorant predation poses a significant challenge to fish farms, as it can lead to considerable losses. This issue has been extensively documented, particularly in catfish farms, where Engle et al. (2021) reported the severe impact of cormorant (*Nannopterum auritus*) predation on production efficiency and profitability. Despite that, the survival rate observed in this study is consistent with those described by Woynárovich and Van Anrooy (2019) for tambaqui production in ponds, where fish of 25-50 g typically achieve survival rates of 60-80%. As curimba is a demersal fish that swims close to the bottom, it was not predated by cormorant and thus, its survival averaged 97%.

The final individual mass of curimba in our study was approximately 145 g in 7 months of culture. Della Rosa et al. (2016) producing *Prochilodus lineatus* integrated with *pacu Piaractus mesopotamicus* in different proportions (*P. lineatus/ P. mesopotamicus*: 1/3, 1/2, and 1/1) with a total density of 0.5 fish per m², harvested curimba with 425 g on average after 11 months. In fed monoculture treatment, curimba reached a final weight of approximately 210 g, indicating that the presence of pacu led to much higher growth than feeding. In our study, we stocked a proportion of 4 tambaqui to 3 curimba, but, because of the tambaqui mortality, we finalized the culture with a proportion of 3 tambaqui to 5 curimba and a total density of approximately 0.5 fish per m². As curimba is a detritivorous fish that eats dead organic matter (Kalous et al. 2012) and was not directly fed in the present study, the higher density of curimba in relation to tambaqui may have reduced food availability for curimba, which, combined with 3 months less culture, led to lower final mean mass. Beyond that, the curimba yield in the current study (550 kg $ha^{-1}$ in 7 months) was about 40% higher than that observed by Della Rosa et al. (2016) (400 kg $ha^{-1}$ in 11 months), when *P. lineatus* was integrated with *P. mesopotamicus*, in all different proportions. Given this context, further studies are needed to evaluate different species proportions and stocking densities to enable curimba to reach the commercial size of approximately 600 g within the specified period.

The inclusion of curimba in the integrated culture did not affect tambaqui FCR, suggesting that curimba growth was sustained by nutrients from aquatic biota, feed wastes and tambaqui feces, as it is a detritivorous fish that eats dead organic matter (Kalous et al. 2012). Franchini et al. (2020) also did not observe a negative impact of the inclusion of curimba in tambaqui FCR in juvenile production. In polyculture systems, the FCR of the pond systems tends to increase when the added species also consumes formulated feed, as observed by Mansour et al. (2021), producing striped catfish, *Pangasianodon hypophthalmus*, and Nile tilapia, *Oreochromis niloticus*. However, the addition of detritivores or benthic species in the integrated culture did not affect the FCR or may even decrease the total FCR of the pond system (Dantas et al., 2020; Della Rosa et al., 2016; Franchini et al., 2020; Junior et al., 2012). The tambaqui FCR was lower than that reported by Lima et al. (2024a) and Lima et al. (2025) for the grow-out phase in monoculture (2.04 and 1.93, respectively), possibly due to the shorter culture period in our study, which may have led to more efficient feed conversion. Additionally, the inclusion of curimba led to a 25% increase in fish biomass in integrated ponds. Thus, the inclusion of curimba in the pond system increases fish biomass production without additional diet inputs, enhancing nutrient recovery. This aligns with the principles of ecological intensification in restorative aquaculture (Aubin et al. 2019; Alleway et al. 2021). This outcome also supports the IMTA principles, where the by-products (organic and inorganic wastes) of one cultured species are recycled as nutritional inputs for others, utilizing species with complementary ecosystem functions (Boyd et al. 2020; Knowler et al. 2020). The growth of the new species is sustained by utilizing nutrients that would otherwise be wasted in the ponds. By converting these nutrients into fish biomass, IMTA increases fish production without requiring additional inputs or expanding the farming area. Consequently, integrated culture contributes to circularity in aquaculture (Checa et al. 2024).

Physical and chemical water parameters were within the recommended range for tropical fish species, according to Boyd et al. (2020), during the entire experiment. The presence of curimba significantly increased suspended and

dissolved solids and turbidity, while chemical variables generally were unaffected. A slightly lower dissolved oxygen in the morning, higher afternoon pH and total nitrogen were observed in the integrated system. The observed effects may be due to the higher total fish biomass in these ponds and/or bioturbation caused by curimba, which resuspend sediments, increasing organic matter decomposition, consuming oxygen and liberating carbon dioxide (which increases the pH) and nitrogen.

The inclusion of curimba in the integrated culture impacted nutrients and plankton dynamics. Higher turbidity and total dissolved and suspended solids associated with lower water transparency observed in the integrated culture were likely due to the bioturbation of the sediment caused by the benthivore behavior of the curimba (Oliveira Junior et al. 2019). This bioturbation suspended the nutrients accumulated on the pond bed, triggering the bottom-up trophic cascade, altering the phytoplankton community, and affecting the entire ecosystem upward through higher trophic levels. This was characterized by the significant increase in chlorophyll concentration in the water column, which was about 80% higher in the integrated culture. Although phytoplankton density was also higher, the high variability of the data may have reduced the statistical power needed to detect significant differences. In addition, the biomass of phytoplankton may be rapidly consumed by the zooplankton, which were significantly higher in the ponds with curimba. Probably, the cladocerans were rapidly consumed by tambaqui, which has a strong preference for this group (Lima et al. 2024a). This may explain their low density; on the contrary, rotifers and copepods were subjected to low predation, and their densities substantially increased in the integrated culture. Therefore, the nutrients that were inactive on the bottom were recovered by the biota and ultimately partially incorporated by the tambaqui. Therefore, curimba has an important role in making nutrients available in the water column, thereby facilitating nutrient recycling in pond systems, making them available to animals of higher trophic levels via food web.

Integrated aquaculture has the potential to positively impact animal health by reducing the prevalence and abundance of parasites (Hosseini Aghuzbeni et al. 2016; Lecocq et al. 2024). For instance, Hosseini Aghuzbeni et al. (2016) demonstrated that the presence of mullets (*Mugil cephalus*) in polyculture systems significantly lowered protozoan parasites such as *Zoothamnium sp.* and *Epistylis sp.* in the gills of whiteleg shrimp *Litopenaeus vannamei*. However, in our study, integrated culture led to a higher mean abundance of monogeneans in tambaqui. As these monogeneans are stenoxenous (parasitizing a single or very closely related species) (Tavares-Dias et al. 2022), they do not parasitize curimba. We hypothesize that this increase may be linked to changes in water quality parameters—such as elevated levels of total dissolved and suspended solids, turbidity, and phytoplankton populations—caused indirectly by the presence of curimba. These environmental shifts could have influenced the life cycles of monogeneans or enhanced their ability to infect hosts. Supporting this, recent meta-analyses show that eutrophic conditions are associated with increased monogenean abundance (Gilbert and Avenant-Oldewage 2021). In general, parasitism intensity is strongly correlated with water quality factors, including organic matter and eutrophication levels (Blanar et al. 2009; Hosseini Aghuzbeni et al. 2016). The evaluation of fish welfare in polyculture is an important way to evaluate the compatibility between the species, evaluating if any negative impact may occur with the integration of the species (Lecocq et al. 2024). In the integrated culture of tambaqui, the inclusion of curimba led to an increase in parasite infestation. While this did not result in fish mortality or impede growth, it is important to address this issue to ensure optimal fish welfare and health.

The mean monogenean abundance observed in both monoculture and integrated culture was higher than the values reported by da Silva et al. (2022), who studied tambaqui (110 g, 3.9–17.1 cm) from a commercial farm, and by Maciel and Affonso (2021), who examined juvenile tambaqui (33 g, 205.33 ± 46.81 cm) in ponds. This difference may be attributed to the larger size of the fish analyzed in the present study (~1700 g). A gradual increase in monogenean abundance in fish

gills is expected over the rearing period, as these parasites have a direct life cycle, reproduce rapidly, and spread efficiently in high-density aquaculture environments. Maintaining parasite loads at manageable levels is essential for preventing potential long-term impacts on fish performance and overall system sustainability.

Integrated multi-trophic aquaculture is an innovative approach to producing fish more sustainably. It enhances the farm's assimilative capacity, reduces the environmental impact of aquaculture, and simultaneously increases farm profits, aligning with the principles of a circular economy (Knowler et al. 2020; Checa et al. 2024). Despite our study did not assess the effects of the integrated system on the surrounding environment, it is evident that the inclusion of curimba contributes to nutrient recovery, which would otherwise become negative environmental externalities. Since no additional nutrients were introduced to the pond system, such as formulated feed, curimba production was fully sustained by nutrients that would have been released into the environment or accumulated on the pond bottom. This indicates that integrating curimba with tambaqui production offers environmental benefits by reducing the nutrient load to produce more fish. Furthermore, the IMTA system was more efficient at converting nutrients into animal biomass, generating economic value, leading to an increase in total income of US$ 1,777.42 while costs increased only US$ 296.28 per hectare per year. This demonstrates that IMTA also enhances farm profitability as stated by Knowler et al., (2020). Producing more fish without increasing formulated feed inputs also reduces the indirect land required for aquaculture, as land is needed to produce the ingredients for fish feed.

The bottom line of a partial budget is the change in net benefit, which indicates whether net returns would increase or decrease with the proposed change and by how much (Engle 2010). The addition of curimba in the monoculture of tambaqui leads to a net benefit gain of US$ 1,481.14 per hectare per year. Production costs increased by US$ 296.28 per hectare per year, mainly due to the cost of purchasing curimba juveniles, which was largely compensated for the increase in revenue. In polyculture systems, cost increases are commonly

linked to the acquisition of additional juveniles, similar to observed in our study, and, in some cases, higher feed consumption (Engle, 2010; Bessa Junior et al., 2012; Mansour et al., 2021). In our study, the inclusion of curimba did not increase the feed input. An increase in income from IMTA systems has been observed for the culture of tambaqui and Amazon River prawn (*Macrobrachium amazonicum*) in the subtropical region (Dantas et al. 2022) and for other species, whether evaluating only the financial aspects from the farm's perspective or also considering external costs and benefits (Ibrahim and Naggar 2010; Knowler et al. 2020). The benefits observed in our study indicate that integrating curimba into the tambaqui production system is economically advantageous and environmentally more sustainable. Curimba appears to be compatible and complementary to tambaqui, making them suitable for co-production (Lecocq et al. 2024). Given that our study was conducted under conditions similar to those of commercial farms, the results are directly applicable in the production sector.

**Conclusions**

Results indicated that the inclusion of curimba in tambaqui culture did not negatively affect the tambaqui growth, survival, productivity or feed conversion ratio. This increased the harvested fish biomass by 25% and it likely reduced the total FCR, improving the use of feed. The inclusion of curimba primarily led to changes in the physical parameters of water due to sediment bioturbation caused by its feeding behavior. This bioturbation likely released nutrients into the water column, stimulating a bottom-up trophic cascade, allowing for the recovery of these nutrients as biota within the pond system. Additionally, the changes in water quality parameters in the integrated culture possibly led to an increase in parasite abundance. Implementing the integrated culture, increased net profit by US$ 1,481.14 per hectare per year. Therefore, tambaqui can be successfully farmed in integrated multi-trophic systems with benthic species, enhancing aquaculture production efficiency and aligning with the principles of a circular economy. Further studies should explore other proportions of tambaqui and

curimba and the stocking densities of each species in the integrated system to optimize curimba growth and total productivity.

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## Manuscrito 2

Este manuscrito foi submetido a *Aquaculture* e está em avaliação.

# Effect of fish biomass on trophic interactions and food assimilation in integrated multitrophic culture of tambaqui (*Colossoma macropomum*) and curimba (*Prochilodus lineatus*)

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## Abstract

Determining the optimal combination and proportion of species remains a key challenge for balancing and optimizing nutrient recycling in Integrated Multi-Trophic Aquaculture (IMTA). The aim of this study was to evaluate the diet, food source utilization and trophic interactions of tambaqui (*Colossoma macropomum*) reared either in monoculture or IMTA with curimba (*Prochilodus lineatus*), using carbon and nitrogen stable isotopes and Ecopath modeling. Three production systems were compared: tambaqui monoculture (T), and two IMTA combining curimba and tambaqui at high (4 t $ha^{-1}$) (TC) and low biomass (1.7 t $ha^{-1}$) (TLC). Across all treatments, tambaqui production was primarily supported by feed (<85%), while curimba growth relied on live or dead zooplankton. However, in TLC, the contribution of zooplankton to the curimba diet significantly decreased (from 68% in TC to 44% in TLC), while formulated feed increased (from 5% in TC to 24% in TLC), resulting in an overlap of isotopic niches between both fish species. IMTA promoted higher ecotrophic efficiency of both feed and zooplankton, as well as a higher system omnivory and Finn's cycling index. IMTA may promote better utilization of natural food resources in the ponds and lead to higher yields compared to monoculture. Maintaining a high tambaqui biomass in IMTA minimizes diet overlap with curimba for formulated feed, while enhancing nutrient recovery and overall system efficiency.

**Keywords**: ecological intensification, Ecopath modelling, integrated culture, low trophic level species, stable isotope, polyculture

## Introduction

Monoculture based on ever-increasing intensification is largely practiced throughout the world aquaculture (Boyd et al. 2020; Thomas et al. 2021), despite the well-established knowledge that it often results in inefficient use of natural resources and a high discharge of nutrients into the environment (Thomas et al. 2021; Lecocq et al. 2024). Integrated multitrophic aquaculture (IMTA) has been proposed as a strategy to enhance the sustainability of the aquaculture sector by improving resource utilization and nutrient recovery in the form of farmed biomass (Rahman et al. 2006; Chopin et al. 2012; Boyd et al. 2020; Lecocq et al. 2024; Chary et al. 2025). Here, we define IMTA following (Boyd et al. 2020) and FAO (2025), as a system in which species occupying different trophic niches are cultured together in appropriate proportions to optimize resource use, minimize environmental impacts, and promote nutrient recycling and sustainability through the integration of fed species with organic and/or inorganic extractive species. One of the main challenges in IMTA is selecting species that are compatible for co-cultivation without negative interactions, while being complementary in resource utilization. Species should occupy different trophic niches to maximize the use of available resources. However, compatibility and complementarity can be influenced by biological and ecological processes, such as variations in species proportions (Thomas et al. 2021; Lecocq et al. 2024).

Knowledge of trophic interactions is essential for designing IMTA systems. By understanding how species interact and their roles at different trophic levels, we can optimize resource utilization (Granada et al. 2018). This optimization enhances energy transfer, strengthens ecosystem resilience, improves water quality, and promotes sustainable practices (Gamito et al.,2020). Consequently, carefully selected species combinations, reflecting these trophic relationships, lead to increased productivity and profitability through reduced input requirements. Several methods have been used to assess these trophic relations, with isotopic analysis and ecological modeling being the primary tools employed. The use of carbon and nitrogen stable isotopes as a tool to quantify the dietary contribution of different food sources to fish growth has provided valuable insights into how fish species utilize various food resources in both natural environments and aquaculture production systems (Narimbi et al., 2018; Zhou and Gu,2020). It is a powerful tool, especially in IMTA systems, where multiple species may compete for the same food sources.

Fish species in aquaculture systems may have their food source utilization influenced by the type of production system and the access to formulated feed. According to Nahon et al. (2020), carp (*Cyprinus carpio*) and roach (*Rutilus rutilus*)

exhibited a dietary shift based on aquaculture intensity. In semi-intensive ponds, carp depended on feed, while roach consumed both feed and natural food. In contrast, both species utilized different natural food resources in extensive systems. Nahon et al. (2024), expanding their study to include rudd (*Scardinius erythrophthalmus*) and perch (*Perca fluviatilis*), observed similar dietary shifts. Rudd followed the pattern seen in carp and roach, while perch relied on natural food in both systems, exploring new food sources in extensive ponds. Lima et al. (2024a) observed a dietary shift in tambaqui (*Colossoma macropomum*) monoculture, with a greater reliance on zooplankton as a natural food source in ponds compared to cages. Other factors, such as the availability of natural food resources and fish density, can also influence food source utilization in aquaculture systems (da Costa et al. 2024; Lima et al. 2024b; Nahon et al. 2024; Rahman et al. 2006). In general, access to formulated feed reduces fish reliance on natural food sources, while the availability of natural food also influences their dietary preferences (Rahman et al. 2006; Nahon et al. 2024; Lima et al. 2024a, b). Changes in production systems and fish density impact natural food availability and water quality parameters, affecting the ecological dynamics of aquaculture ecosystems. Although formulated feed supports increased fish productivity, it can lead to nutrient and organic matter overloaded in pond systems, resulting in changes to the trophic structure and subsequent trophic interactions (Aubin et al. 2021).

Optimizing IMTA systems depends on defining the ideal species proportions, which requires assessing their impact on trophic interactions within the ecosystem (Thomas et al. 2021). Ecosystem models provide a comprehensive framework for assessing species dynamics, allowing changes to be examined in the context of the entire system (Heymans et al. 2004). The Ecopath, originally developed for marine ecosystems, is a nutrient balance model based on the ecosystem approach. It describes energy flow and material cycling according to the principles of nutrient dynamics, helping to understand the structure and function of aquatic ecosystems (Christensen et al. 2024). It is implemented in a free software Ecopath with Ecosim (EwE). Ecopath models have been applied to many aquatic ecosystems, but their use to evaluate pond aquaculture is yet limited, despite their successful application (Aubin et al. 2021; Dong et al. 2021; Feng et al. 2018; Gamito et al. 2020; Xiao et al. 2024; Xu et al. 2011; Zhang et al. 2024). Understanding the complex trophic interactions in pond ecosystems makes it possible to adapt the species assemblage as a function of the pond´s natural productivity and biodiversity (Aubin et al. 2021). This is even more relevant in IMTA systems, which combine different aquaculture species within an existing ecosystem that already hosts intrinsic communities such as bacteria,

phytoplankton, zooplankton, and benthic macro-invertebrates (Thomas et al. 2021).

Integrated aquaculture is one of the oldest aquaculture production systems and offers greater efficiency by optimizing resource utilization and recycling nutrients from farmed biomass, but it has been largely overlooked in favor of intensive monoculture in Western aquaculture development (Amoussou et al. 2022). The most studied integrated system is carp polyculture, which has been practiced in China since the 7th to 9th centuries (Milstein 1992). However, carp species are not ideal candidates for western aquaculture due to low consumer acceptance and market value (Thomas et al. 2021). In Brazil, for instance, carp polyculture has been practiced for the past four decades but remains a minor contributor to national aquaculture production, as its consumption is largely regional (Valenti et al. 2021). Thus, leveraging integrated culture to enhance aquaculture sustainability requires identifying alternative species for the integrated culture, especially those that complement traditional monoculture and encourage industry adoption. Considering this, we proposed an IMTA system combining tambaqui (*Colossoma macropomum*), an omnivorous fish that primarily feeds on zooplankton in aquaculture systems and is the main native species farmed in Brazil (Valenti et al. 2021; Lima et al. 2024a), with curimba (*Prochilodus lineatus*). Curimba, also known as curimbatá or curimatã, is a benthic, detritivorous species with a long tradition in Brazilian aquaculture, which has recently been produced in Vietnam and China, making it an ideal candidate for IMTA systems (Kalous et al. 2012; Valenti et al. 2021). The integration of these species aligns with the concept of intrinsic compatibility discussed by Thomas et al. (2021), which supports the IMTA culture of benthic and pelagic species to reduce interspecific competition. This species combination also fits the definition of Level 1 integrated aquaculture, as proposed by Boyd et al. (2020), who classify integration levels based on the ecological interactions among co-cultured species. In Level 1 systems, species are raised together in the same facility but occupy distinct ecological niches, meaning they rely on different resources or perform complementary functions within the system. As the biomass of different species in the production system can influence their access to food resources (Rahman et al. 2006; Azim and Little 2006), this parameter was also assessed in the present study. Accordingly, the impact of incorporating a benthic species into tambaqui monoculture, as well as the effect of fish biomass in IMTA system, were assessed using two complementary methods—stable isotope analysis and Ecopath modeling—to investigate differences in feeding behavior and their consequences for ecosystem quality and circularity.

## Material and methods

*Study site and pond preparation*

The study was conducted at the Aquaculture Experimental Center of the Brazilian Agricultural Research Corporation (Embrapa) in Palmas, Tocantins, Brazil (10°8'1.33"S, 48°19'9.86"W) from July 2023 to February 2024. Tambaqui fingerlings (2.0 ± 0.8 g) and curimba (3.2 ± 2.1 g) were purchased from commercial hatcheries located in Brejinho de Nazaré, TO, Brazil (48°35′17.93″S, 11°1′52.95″W), and Ipueiras, TO, Brazil (48°27′54.274″S, 10°58′14.88″W), respectively. The fish were maintained in ponds and fed until apparent satiety four times a day with extruded commercial feed (pellet size 1–2.6 mm, 45% crude protein) until they reached the initial size required for the study: 60-day-old tambaqui (29 ± 4 g) and 60-day-old curimba (15 ± 9 g). Prior to the experiment, ten ponds, each measuring 600 m² with a depth of 1.3 m, were prepared by draining and disinfecting with quicklime (200 g $m^{-2}$). The ponds were subsequently limed with limestone (200 g $m^{-2}$) and fertilized using urea (5 g $m^{-2}$), simple superphosphate (3 g m-²), and rice bran (10 g $m^{-2}$). After preparation, the ponds were filled with water sourced from a local dam. During the 7 months of the study, additional water was added only to offset evaporation and seepage losses.

*Experimental design*

Two production systems (treatments) were stocked: (T) a monoculture system with tambaqui stocked at a density of 0.4 fish $m^{-2}$, with three replicates; and (TC) an IMTA culture combining tambaqui (0.4 fish $m^{-2}$) and curimba (0.3 fish $m^{-2}$), with seven replicates. The stocking densities corresponded to those currently used in the commercial grow-out phase of tambaqui (Valenti et al. 2021). However, cormorant predation during the second and third months of the experiment significantly reduced the number of tambaqui in four of the TC ponds, leading to lower final biomass values. As the biomass of different species in the production system can influence their access to food resources (Azim and Little 2006), treatments were reclassified after harvest according to the final tambaqui biomass: ponds with biomass >3.5 t $ha^{-1}$ were grouped as TC (three ponds) (0.25 fish $m^{-2}$), while ponds with biomass <2.0 t $ha^{-1}$ were grouped as TLC (four ponds) (0.1 fish $m^{-2}$). Thus, the study included three replicates for T, three for TC, and four for TLC. In T, TC, and TLC, the final biomass of tambaqui was 3.8 t $ha^{-1}$, 4.1 t $ha^{-1}$, and 1.7 t $ha^{-1}$, respectively. The experiment lasted seven months, corresponding to the time required for tambaqui to reach market size.

The target species was tambaqui, which was fed twice daily (8:00 h and 16:00 h) with commercial extruded feed (Archer Daniels Midland Company, Brazil).

Feeding protocols were adjusted according to fish weight: for weight ranges of 30–230 g, 230–410 g, and 410–700 g, the fish were fed a diet containing 32% crude protein at feeding rates of 4.5%, 3%, and 2% of body weight per day, respectively. For weight ranges of 700–1130 g and above 1130 g, the fish were fed a diet containing 28% crude protein at feeding rates of 2% and 1.5% of body weight per day, respectively (Oliveira et al. 2013). Feeding was over when the fish ceased eating or when the daily dose computed by feed rate was reached. The actual quantity of feed provided was recorded; the amount of uneaten feed floating on the pond surface was negligible, so the total feed supplied served as a reliable proxy for actual feed intake. Each month, 30 tambaqui were randomly selected from each pond, weighed, and their biomass was used to adjust the amount of feed offered. After weighing, the fish were returned to their respective ponds. The apparent feed conversion ratio (FCR) was calculated using the formula: FCR = total feed supplied / total net weight gain during the experiment. At the end of the experiment, the ponds were drained, and all fish were harvested and weighed in batches of five, with the total yield recorded. Productivity was determined by calculating the ratio of final biomass to pond area. The specific growth rate (SGR) was calculated using the formula: SGR = 100 x (ln final weight - ln initial weight) / time of production (days).

*Water quality parameters*

Temperature, dissolved oxygen, and pH were monitored three times a week at 08:00 a.m. using a YSI Professional Plus probe (Yellow Springs Instruments Company, Yellow Springs, USA). Each pond was sampled monthly for the analysis of chlorophyll-*a*, total suspended solids, dissolved solids, and turbidity. These parameters were measured following the APHA methodology (APHA 2017), except for turbidity, which was analyzed using a turbidimeter (Hach 2100Q, Loveland, CO, USA).

*Sample collection for carbon and nitrogen stable isotope analysis*

The food consumed by tambaqui in aquaculture ponds has been previously described through stomach content studies (Gomes and Silva 2009b; Lima et al. 2024a). This information guided the selection of possible food sources collected for this species. To investigate the food assimilated by tambaqui, samples were collected from tambaqui muscle, commercial feed, zooplankton, aquatic insects, benthic macro-invertebrates, and sediment organic matter. In contrast, there is no information available regarding the natural food items ingested by curimba in aquaculture ponds. Therefore, we considered the dietary items consumed by this species in its natural environment (Bayo and de Yuan 1996; Fugi et al. 2001). Thus, to investigate curimba’s food assimilation, samples were collected from

curimba muscle, commercial feed, zooplankton, benthic macro-invertebrates, sediment organic matter, and tambaqui feces.

Sampling occurred at the end of the 7-month experiment across all ten ponds. From each pond, five specimens of each fish species were euthanized using a eugenol bath (35 mg l-1) (Roubach et al. 2005). After euthanasia, white dorsal muscle tissue was dissected from above the lateral line of each fish. A sample from each bag of formulated feed was combined into a composite sample, from which one subsample was taken for isotopic analysis. Zooplankton was sampled from each pond using a 68 µm mesh net, which was pulled horizontally through the water twice. The samples were then pooled into a composite sample, from which a subsample was taken for isotopic analysis. To sample benthic macro-invertebrates (Bent), one basket (0.06 m2) containing 500 g of dry soil from the relevant pond was placed at the bottom of each pond and left to be colonized by benthic organisms over a two-month period. At the end of the period, the sediment from the basket was first sieved through a 1000 µm mesh to remove debris and stones, followed by a 250 µm mesh to eliminate finer sediment particles. Benthic organisms larger than 250 µm were then extracted from the remaining material. For each pond, all benthic organisms found were grouped in a unique sample. Insects found during the sampling of both zooplankton and benthic organisms were collected. As only few insects were sampled from each pond, they were pooled into a composite sample containing insects from all ponds. Most of the insects in this composite sample were Odonata larvae. Sediment organic matter was collected using sediment cores (19.63 cm² surface area, and ~ 15 cm depth) from three locations in each pond and pooled into a composite sample per pond. The sediment was then sieved through a 1mm mesh to remove debris and stones, after which a subsample was taken for isotopic analysis. Feces were collected by applying cephalocaudal pressure to the fish's abdominal region, starting 10 cm from the anus. This procedure was performed on five fish per pond, and the fecal samples from each pond were pooled into a composite sample. All samples were dried in an oven at 60°C for 72 hours and stored at 4°C until analysis.

*Carbon and nitrogen stable isotope analysis*

The isotopic analyses were carried out at the Stable Isotopes Center of the Universidade Estadual Paulista (UNESP, Brazil). Each sample was homogenized individually in a cryogenic mill at -196°C and then weighed in tin capsules (~0.5 mg). The $\delta^{13}C$ and $\delta^{15}N$ values of samples were determined via elemental analyzer/isotope ratio mass spectrometry in continuous-flow mode using an isotope ratio mass spectrometer (Delta V, Thermo Scientific) coupled to an elemental analyzer EA (Flash 2000, Thermo Scientific) through a gas interface

(ConFlo IV, Thermo Scientific). The isotopic ratios R ($^{13}C/^{12}C$) or R ($^{15}N/^{14}N$) were expressed in conventional isotope delta (δ) in per mil (‰) relative to the levels of $^{13}C$ in Vienna Pee Dee Belemnite and $^{15}N$ in the atmosphere, respectively. The standard uncertainty of the simultaneous measure was estimated at ± 0.15‰ and ± 0.10‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Values of $\delta^{15}N$ and $\delta^{13}C$ were calibrated according to international standards USGS61, USGS62 and USGS63.

*Stable isotope mixing models and standard ellipse areas*

The contributions of food sources to fish assimilation were estimated using Bayesian stable-isotope mixing models (*simmr* package; Parnell et al., 2013) of R software v. 4.3.1 (R Core Team 2025). The C and N trophic-discrimination factors were estimated at 1‰ and 3.5‰, respectively, for both tambaqui and curimba based on Britton and Busst (2018). The Bayesian standard ellipse area ($SEA_B$) occupied by a species in $\delta^{13}C$ and $\delta^{15}N$ spaces was calculated for tambaqui and curimba reared in T, TC and TLC using the *SIBER* package (Jackson et al. 2012) of R. The $SEA_B$ metric is a bivariate equivalent of the standard deviation that contains the mean isotopic niche of the core population. $SEA_B$ metrics were chosen instead of convex hull metrics (Layman et al. 2007), which include outlier individuals, due to their robustness to differences in sample size and to circumvent the bias caused by small sample sizes. $SEA_B$ sizes and overlap between species and treatments were then compared using a Bayesian approach based on Markov-chain Monte Carlo (with 100 000 draws) (Jackson et al. 2012). We calculated the proportion, and thus probability, that the $SEA_B$ for one group was smaller than that for another group.

*Trophic modeling using Ecopath*

The trophic interactions and energy fluxes of tambaqui monoculture and IMTA of tambaqui and curimba were modelled using the Ecopath with Ecosim free software (EwE) (www.ecopath.org) that provides a static description of an ecosystem at a precise period of time (Christensen et al. 2024). For each model, the average values from the replicates of each treatment were used as input data. The model represents the ecosystem through functional groups that collectively account for all energy flows, ensuring a balanced representation. The energy of each functional group should maintain a balance between input and output, i.e., production - predation mortality - harvesting - net migration - biomass accumulation - other mortality = 0 (Christensen et al. 2024).

This study categorized the ecosystem in the tambaqui monoculture (T) into six functional groups: phytoplankton in the producer category; zooplankton, benthos, and tambaqui in the consumer category; and formulated feed and detritus

in the detritus category. In the IMTA systems (TC and TLC), a seventh group, curimba, was added to the consumer category. Formulated feed was categorized as part of the detritus functional group because it is neither a producer nor a consumer but supports the food web by serving as a food source (Bayle-Sempere et al. 2013). Unlike isotopic models, the Ecopath model did not include insects and tambaqui feces as separate functional groups due to insufficient data for calculating the biomass of these food sources within the pond system. Given the small size of the ponds and the duration of the study (1 production cycle = 7 months), the units used throughout this work were g $m^{-2}$ and g $m^{-2}$ 210 $days^{-1}$.

The Ecopath model requires basic data, including estimates of biomass (B), production-to-biomass (P/B) ratios, consumption-to-biomass (C/B) ratios for each trophic group, and diet compositions (Christensen et al. 2024). A system of linear equations was

established, incorporating these three parameters, with only the Ecotrophic Efficiency (EE) estimated by the model (1):

$$Pi = Yi + BiM0i + Ei + BAi + Pi(1 - EEi) \text{ (1)}$$

where, $P_i$ is the total production rate of (i), $Y_i$ is the total fishery catch rate of (i), B*i* the biomass of group (i), $M_i$ is the total predation rate for group (i), E*i* is the net migration rate (emigration–immigration), $BA_i$ is the biomass accumulation rate for (i), while $M0_i = P_i(1 - EE_i)$ is the "other mortality" rate for (i).

The equation can also be re-expressed as (2 and 3),

$$Bi \times \left(\frac{P}{B}\right) i \ \sum_j Bj \ \times \left(\frac{Q}{B}\right) j \ \times DCji - \left(\frac{P}{B}\right) i \ \times Bi \ (1 - EEi) - Yi - Ei - BAi = 0 \text{ (2)}$$

$$Bi \times \left(\frac{P}{B}\right) i \ \times EEi - \sum_j Bj \ \times \left(\frac{Q}{B}\right) j \ \times DCji - Yi - Ei - BAi = 0 \text{ (3)}$$

where $(P/B)_i$ is the production/biomass ratio, $(Q/B)_j$ is the consumption/biomass ratio of predator, $DC_{ji}$ is the fraction of prey (i) in the average diet of predator (j), and $EE_i$ is the ecotrophic efficiency of (i), which can be described as the proportion of the production that is utilized in the system.

In this study, B, P/B, and C/B were calculated for each experimental unit, and the average values for each treatment were used in the model. The biomass in the models used dry weight values for all functional groups. Fish biomass was calculated using the formulae (4 to 7) described in Aubin et al. (2021):

$$Fish\ Biomass\ (g) = Nb\ x\ Wm \text{ (4), where:}$$

$$Wm = \frac{Initial\ fish\ weight\ (g) + Final\ fish\ weight\ (g)}{2} \text{ (5);}$$

$$Nb = \frac{Initial\ number\ of\ fish - Final\ number\ of\ fish}{Z} \text{ (6);}$$

$$\text{and } Z = -\ln\left(\frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish}\right) \text{ (7).}$$

The dry biomass of the fish was used in the analysis, with dry biomass estimated as 25% of the fresh biomass, following the recommendation of Cavali et al. (2021).

P/B ratios were derived using the equations from Christensen et al. (2005) (8 and 9):

$$Fish\ \left(\frac{P}{B}\right) = \frac{Bacc}{B} + Z \text{ (8), where:}$$

$$Bacc = \frac{Fish\ final\ biomass\ (g)}{Fish\ initial\ biomass\ (g)} \text{ (9),}$$

B represents fish biomass, and Z corresponds to the same value used in the fish biomass calculation.

The C/B ratio for curimba was calculated using the formula provided by Christensen et al. (2005) (10):

$$\log(C/B) = 5.847 + 0.280 \log Z - 0.152 \log Winf - 1.360\ T + 0.062\ A + 0.510\ h + 0.390\ d, \text{ (10)}$$

where Z is the same value used to calculate fish biomass, Winf is the asymptotic weight and A is the aspect ratio of caudal fin, both obtained for *Prochilodus lineatus* from the FishBase website (Froese and Pauly 2024), T is the mean of temperature (°K) during the production cycle, h=0 and d=1, considering curimba is a detritivorous species (Kalous et al. 2012).

Feed consumption by tambaqui was estimated using the feed conversion ratio, which was then used to calculate the tambaqui C/B ratio (Gamito et al. 2020). In TLC, tambaqui was given the same feed consumption estimates as in T and TC, since in these treatments it was the primary consumer of formulated feed, as detailed in the Results section.

Phytoplankton biomass was estimated using the average monthly chlorophyll-*a* concentration, assuming that chlorophyll-*a* accounts for 1% of the phytoplankton's dry mass (Reynolds 2006) (11):

$$Phyto\ B\ (g/m2) = \frac{Chlorophyll\ a\ concentration\ (g/m^3)}{0.01}\ x\ pond\ depth\ (m) \text{ (11)}$$

Phytoplankton production in grams of carbon assimilated per m² was calculated using the formula provided by Ryther and Yentsch (1957) (12):

$PP = 3.7\ x\ chlorophyll\ a\ concentration\ \left(\frac{g}{m3}\right) x\ local\ surface\ radation\ (kcal/m2/day)$ (12)

The final value was then converted to total dry weight using the relation proposed by (Reynolds 2006), which states that carbon accounts for 50% of the biomass in phytoplankton.

Zooplankton concentration in the pond was estimated bimonthly using a 68 µm mesh net, which was dragged for 10 meters in each pond, filtering a total volume of 1075 l of water. The zooplankton samples were then quantitatively analyzed using Sedgwick-Rafter chambers. Zooplankton biomass was estimated using the average zooplankton concentration in the pond, combined with the mean dry weight of zooplankton organisms (Dumont et al. 1975). Zooplankton production was calculated using the individual growth rate of zooplankton (Zoo IGR) through the formula provided by Zhou et al. (2010) (13):

$$Zoo\ IGR = 0.033\ \left(\frac{Ca}{Ca+205\ e^{-0.125\,T}}\right) e^{0.09T}\ \ W^{-0.06}\ (13)$$

Where T is the mean temperature (°C) during the production cycle, Ca is the chlorophyll concentration (g C $m^{-3}$), and W is the individual body weight of the zooplankton (g of carbon). To calculate zooplankton production, it was assumed that 50% of the dry weight of zooplankton is composed of carbon (Andersen and Hessen 1991).

Benthos' biomass was calculated based on the abundance of benthic organisms in the pond and the average individual dry weight of each organism. The sampling procedure for benthic organisms described in the *Sample Collection section* was repeated every two months to quantify benthos abundance throughout the production cycle, with the average used in the benthos biomass estimation. After counting the benthic organisms, they were dried, and the average dry weight of the organisms was used to estimate biomass. Benthos production was calculated using the formula described by Morin and Dumont (1994) (14):

$$Bent\ P = -2.09 - 0.27 \log individual\ biomass + 0.025\ T\ (14)$$

Where T is the mean of temperature (°C) during the production cycle.

Since no data on consumption rates for zooplankton and benthic organisms were available, a P/C ratio of 0.3 was used in the model, as suggested by Christensen et al. (2005). A P/C ratio of 0.3 indicates that consumption is about three times higher than production. The total amount of feed distributed in each pond was recorded throughout the production cycle and included as an inert input. Feed was classified as detritus (having a trophic level of 1), as suggested by Bayle-Sempere et al. (2013). To estimate the biomass of other detritus in the pond,

we applied the detritus accumulation formula proposed by Christensen and Pauly (1993) (15):

$$LogD = -2.41 + 0.954 \log PP + 0.863 \log E \quad (15)$$

Where D is the biomass of detritus (g C $m^{-2}$), PP is the phytoplankton production (g C assimilated $m^{-2}$), and E is the depth of the euphotic zone. Since the euphotic zone is typically characterized as 2.5 times the Secchi transparency (Golterman et al. 1978), and this value was greater than the pond depth, we assumed the pond depth (1.3 m) as the euphotic zone for all ponds.

Unassimilated consumption represents the fraction of food that is not assimilated by each functional group and returned to the environment as feces or excreta. The default values suggested by Christensen et al. (2024) and Gamito et al. (2020) were used: 0.4 for zooplankton and benthic organisms, and 0.2 for fish species. Fertilizers were included in the system as detritus, while biomass lost to bird predation was categorized as emigration, based on the average weight of the species during the month in which predation occurred. The tambaqui and curimba biomass at the end of the production cycle was added to the model as biomass accumulation within their respective functional groups.

Ecotrophic Efficiency, as estimated by the Ecopath model, represents the proportion of production that is harvested or predated upon. In the Ecopath model, the initial attempt at balancing the model often involves evaluating whether the EE for each group is less than 1. It is common for the EE of some groups to exceed 1 in the first iteration of the model when using initial input values (Xu et al. 2011). When unbalanced groups were encountered during modeling, we adjusted the diet composition of zooplankton and benthic organisms to achieve mass balance, as their diet composition was not available from isotopic analysis (Christensen and Walters 2004; Dong et al. 2021).

*Ecopath indicators*

The EwE software calculates a pedigree index, which evaluates the quality and uncertainty of the input and output parameters. To estimate this index, parameters directly assessed from experimental data, such as biomass, tambaqui consumption, and P/B ratios for fish and feed, were considered to have high precision. In contrast, other parameters, such as the biomass, P/B and C/B ratios for phytoplankton, zooplankton, benthos, and curimba consumption, were classified as having low precision, as they were estimated to be using indirect methods. For the pedigree index, the diet of tambaqui and curimba was classified based on detailed diet composition studies, while the diet of zooplankton and benthic organisms was classified based on general knowledge.

Several indices were calculated by EwE for this study, as they reflect the balance of aquatic ecosystems, the level of nutrient circularity, the degree of system organization, and the complexity of food webs. They were: (1) total system ascendency, (2) total system throughput, (3) total primary production-to-total respiration ratio, (4) net system production, (5) the Finn cycling index, (6) the connectance index, (7) the system omnivory index, (8) the average path length, and (9) the prey overlap index. All were calculated based on the methods described in Christensen et al. (2005). These indices have also been used in previous studies on freshwater aquaculture (Gamito et al. 2020; Aubin et al. 2021; Xiao et al. 2024).

*Statistical analysis*

Water quality parameters, fish mass, yield, FCR, and $\delta^{13}C$ and $\delta^{15}N$ values were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Bartlett test). Data that did not meet these assumptions were transformed using the Box-Cox method (Box and Cox 1964). Subsequently, data were analyzed using one-way ANOVA, followed by post-hoc Tukey's test for pairwise comparisons. Chlorophyll-*a*, total dissolved solids, the $\delta^{13}C$ value of sediment, and the $\delta^{15}N$ values to compare tambaqui and curimba in TC did not meet the assumptions of normality and/or homoscedasticity, even after transformation, and were analyzed using the non-parametric Kruskal–Wallis test. All statistical analyses and graphics were performed with the free software R, Version 4.2.3 (R Core Team 2025).

*Legal and Ethical Aspects*

The study complied with official Brazilian guidelines for the care and use of animals for scientific and educational purposes (Concea-CEUA protocol 76/2022), and with the National Management System for Genetic Heritage and Associated Traditional Knowledge (AB47B85).

## Results

*Physical, chemical and biological parameters of water*

Temperature, dissolved oxygen and pH did not differ significantly between treatments (Anova test, $p > 0.05$). The mean temperature was 28.9 ± 0.2 °C, dissolved oxygen, 6.2 ± 1.3 mg $L^{-1}$; and pH, 7.9 ± 0.2. Chlorophyll-*a* was higher in TC, while total dissolved solids did not differ between treatments (Table 1). Total suspended solids and turbidity were higher in TC, lower in T, and both did not differ from TLC (Table 1).

Table 1. The mean ± standard deviation of the water quality parameters measured in tambaqui (*Colossoma macropomum*) monoculture (T) and IMTA system integrating curimba (*Prochilodus lineatus*) and tambaqui (*Colossoma macropomum*) cultured at high (TC) and low (TLC) biomass.

| | Treatment | | | *P*-value |
|---|---|---|---|---|
| | T | TC | TLC | |
| Chlorophyll-*a* (mg $L^{-1}$)** | 19.0 ± 27.4 [b] | 34.5 ± 37.2 [a] | 13.9 ± 12.0 [b] | 0.0072 |
| Total Dissolved Solids (mg $L^{-1}$)** | 86.6 ± 41 [a] | 104.7 ± 55.6 [a] | 105.4 ± 52.6 [a] | 0.3906 |
| Total Suspended Solids (mg $L^{-1}$) * | 11.8 ± 10.9 [b] | 25.6 ± 24.7 [a] | 19.1 ± 18.8 [ab] | 0.0235 |
| Turbidity (NTU) * | 28.5 ± 29.3 [b] | 49.0 ± 41.1 [a] | 41.4 ± 39.3 [ab] | 0.0342 |

Different letters in the same row indicate significant differences ($p < 0.05$). *Anova test. **Kruskal–Walli's test.

*Growth performance of fish and feed-conversion ratio*

The final individual weight of tambaqui was not influenced by the inclusion of curimba in TC (Table 2). However, the low tambaqui biomass in TLC resulted in higher individual tambaqui weight. Fish yield was higher in TC and lower in TLC (Table 2). Considering tambaqui eating all feed, the tambaqui FCR in T and TC were similar and was higher in TLC (Table 2). The SGR of tambaqui and curimba, final weight of curimba, curimba biomass, and FCR considering tambaqui and curimba biomasses did not differ between treatments (Table 2).

Table 2. The mean ± standard deviation of the productive performance of tambaqui (*Colossoma macropomum*) monoculture (T) and IMTA system integrating curimba (*Prochilodus lineatus*) and tambaqui (*Colossoma macropomum*) cultured at high (TC) and low (TLC) biomass. FCR – Feed conversion ratio. SGR – Specific growth rate.

| | T | TC | TLC | *p* value |
|---|---|---|---|---|
| *Tambaqui* | | | | |
| Final biomass (kg ha-1) | 3783 ± 383 [a] | 4116 ± 550 [a] | 1729 ± 575 [b] | 0.0016 |
| Final weight (g) | 1693 ± 307 [b] | 1776 ± 194 [b] | 2170 ± 182 [a] | 0.0450 |
| FCR Tambaqui | 1.78 ± 0.18 [b] | 1.78 ± 0.26 [b] | 2.66 ± 0.27 [a] | 0.0056 |
| SGR Tambaqui | 1.93 ± 0.08 [a] | 1.96 ± 0.05 [a] | 2.05 ± 0.03 [a] | 0.0584 |
| *Curimba* | | | | |
| Final biomass (kg ha$^{-1}$) | - | 561 ± 179 | 774 ± 147 | 0.1751 |
| Final weight (g) | - | 144 ± 44 | 182 ± 41 | 0.3194 |
| SGR Curimba | - | 1.06 ± 0.15 | 1.17 ± 0.13 | 0.3359 |
| *Total Yield* (kg/ha) | 3783 ± 383 [ab] | 4683 ± 550 [a] | 2513 ± 625 [b] | 0.0057 |
| *FCR total* | 1.73 ± 0.16 [a] | 1.52 ± 0.23 [a] | 1.95 ± 0.19 [a] | 0.1701 |

Different letters indicate significant differences between treatments ($p < 0.05$).

*$\delta^{13}C$ and $\delta^{15}N$ values of fish and their potential food sources*

The $\delta^{13}C$ and $\delta^{15}N$ values significantly differed between tambaqui and curimba in TLC ($p < 0.05$). Tambaqui were $^{13}C$-enriched and $^{15}N$-depleted compared to curimba by 1.69 and 0.68‰, respectively (Figure 1, Supplementary Table S1). In TC, only the $\delta^{13}C$ values differed between the species ($p < 0.05$), in which tambaqui were $^{13}C$-enriched compared to curimba by 3.27‰ (Figure 1, Supplementary Table S1). The $\delta^{15}N$ values of curimba, as well as the $\delta^{13}C$ and $\delta^{15}N$ values of tambaqui, did not differ significantly between treatments ($p > 0.05$). Curimba showed lower $\delta^{13}C$ values in the TC compared to the TLC ($p < 0.001$).

The $\delta^{13}C$ and $\delta^{15}N$ values of each potential food source did not differ significantly between treatments ($p > 0.05$) (Figure 1, Supplementary Table S1). Feed was the food source with the highest $\delta^{13}C$ values (-17.32‰), while insects had the lowest $\delta^{13}C$ values (-23.77‰) across all treatments (Table 3); While for $\delta^{15}N$ values, zooplankton presented the highest value in all treatments (6.59 - 8.05‰), and sediment the lowest value (2.82 - 3.17‰) (Figure 1, Supplementary Table S1).

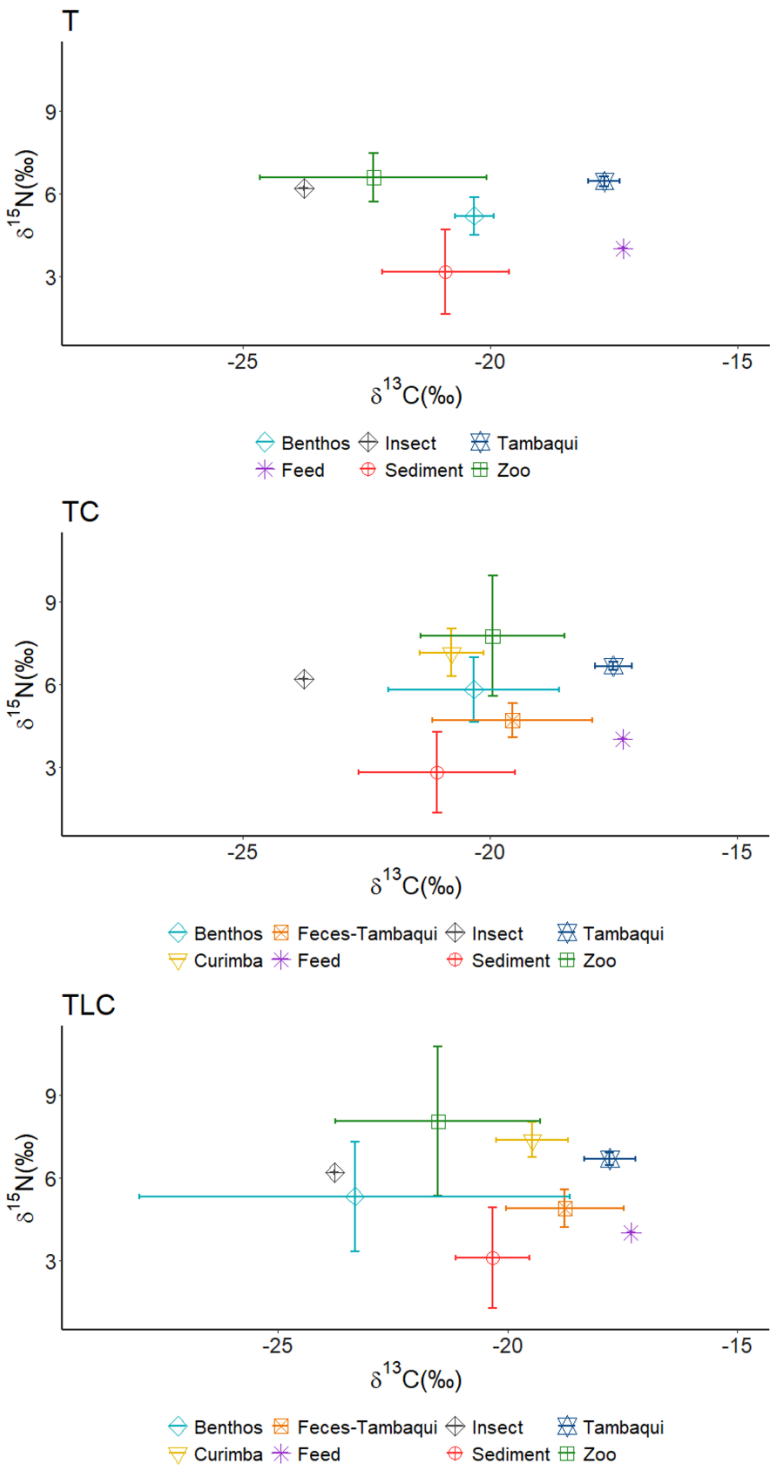


Figure 1. Mean δ¹³C and δ¹⁵N values (± standard deviation) of fish and potential food sources from tambaqui (*Colossoma macropomum*) monoculture (T) and IMTA system integrating curimba (*Prochilodus lineatus*) and tambaqui (*Colossoma macropomum*) cultured at high (TC) and low (TLC) biomass.

The mixing model revealed that commercial feed was the primary food source for tambaqui across all treatments, with no significant differences in the proportions of different food sources between treatments ($p > 0.05$, Figure 2, Supplementary Table S2). In both IMTA and monoculture systems, commercial feed contributed more than 85% to the diet of tambaqui. Compared to commercial feed, tambaqui was $^{13}$C-enriched by 0.19–0.46‰ and $^{15}$N-enriched by 2.43–2.67‰ (Figure 1). Zooplankton accounted for less than 7% of the tambaqui diet, whatever the treatment. The contributions of benthos, insects, and sediment to the tambaqui diet ranged from 2–4%. For curimba, zooplankton was the primary food source used in both TC and TLC (44–68%, Figure 2, Supplementary Table S2). However, the zooplankton contribution significantly decreased (44% *versus* 68%) while formulated feed (25% *versus* 5%) and tambaqui feces (22% *versus* 8%) increased in the TLC compared to TC, respectively ($p < 0.01$). Compared to zooplankton, curimba was $^{13}$C-depleted by 0.83‰ in TC, $^{13}$C-enriched by 2.05‰ in TLC, and $^{15}$N-depleted by ~0.6‰ in both treatments (Figure 1). Standard ellipse areas (SEAc) of curimba and tambaqui did not overlap in TC and showed an overlap of 16% in TLC (Figure 3, Supplementary Figure S1). Direct evidence from stomach content analysis (Supplementary data, Table S3) corroborated the isotopic patterns, with tambaqui showing ingestion of insects, zooplankton, plants, and sediment, while curimba exhibited a predominance of sediment and occasional zooplankton. Although based on a limited number of specimens, these observations qualitatively support the dietary inferences obtained from stable isotopes.

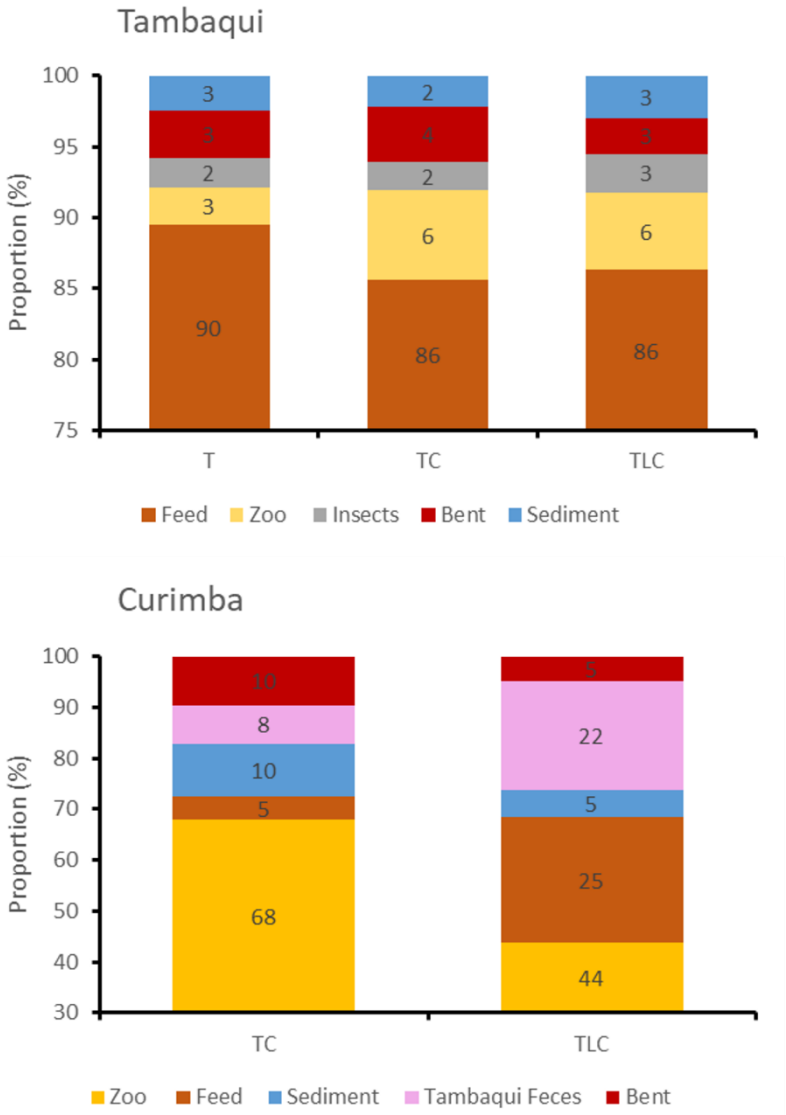


Figure 2. Estimated contributions of food sources to the diets of tambaqui and curimba in tambaqui (*Colossoma macropomum*) monoculture (T) and IMTA system integrating curimba (*Prochilodus lineatus*) and tambaqui cultured at high (TC) and low (TLC) biomass. Zoo = zooplankton; Bent = benthos.

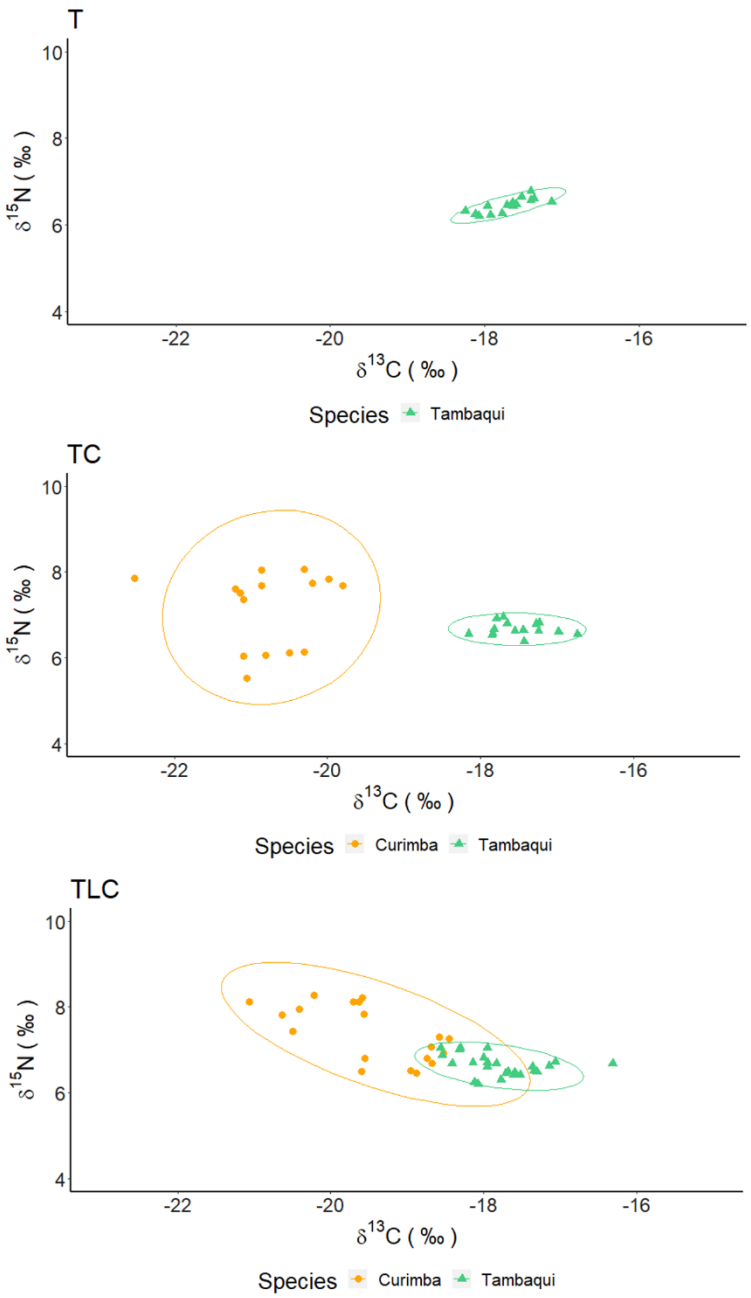


Figure 3. Individual δ¹³C and δ¹⁵N values for tambaqui and curimba in monoculture (T) and in IMTA culture with curimba and tambaqui at high (TC) and low (TLC) biomass. Lines enclose the standard ellipse area (SEAc) for each species in each treatment.

*Results of Ecopath Modelling*

The diet matrix used in Ecopath was partly derived from the results of isotopic analyses, while the remaining portion was constructed to ensure ecosystem balance (Table 3). The diet composition of tambaqui and curimba was based on isotopic analysis, whereas the diet composition of zooplankton and benthos was adjusted to achieve mass balance, as their diet could not be determined through isotopic analysis.

Table 3. Input diet composition matrix (data are in proportion from 0 to 1). The diet of zooplankton (Zoo) and benthic organisms (Bent) was the same in T, TC and TLC. Phyto = Phytoplankton; T = tambaqui (*Colossoma macropomum*) monoculture; TC = IMTA of tambaqui at high biomass and curimba (*Prochilodus lineatus*); TLC= IMTA of tambaqui at low biomass and curimba.

| Prey | Predator/Consumer | | | | | | |
|---|---|---|---|---|---|---|---|
| | Zoo | Bent | Tambaqui in T | Tambaqui in TC | Tambaqui in TLC | Curimba in TC | Curimba in TLC |
| Phyto | 0.500 | - | - | - | - | - | - |
| Zoo | - | 0.100 | 0.050 | 0.083 | 0.081 | 0.679 | 0.439 |
| Bent | - | - | 0.030 | 0.039 | 0.250 | 0.097 | 0.048 |
| Feed | - | 0.100 | 0.895 | 0.856 | 0.863 | 0.046 | 0.245 |
| Detritus | 0.500 | 0.800 | 0.025 | 0.022 | 0.031 | 0.178 | 0.268 |

The prey overlap index from Ecopath modeling between curimba and tambaqui increased from 16.6% in TC to 47.5% in TLC. The prey overlap index between curimba and zooplankton increased from 17.7% in TC to 32.4% in TLC, while overlap between curimba and benthic organisms rose from 36.9% in TC to 57.3% in TLC.

Zooplankton EE was highest in TC, lowest in T, and intermediate in TLC, while benthic organism EE remained low across all treatments. Detritus EE was high in all treatments. Feed EE was higher in IMTA systems in comparison with monoculture system (Table 4). Tambaqui and curimba exhibited a high EE and the values were quite similar across the treatments (Table 4).

Table 4. Basic input and calculated parameters for Ecopath model of tambaqui (*Colossoma macropomum*) monoculture (T) and IMTA system integrating curimba (*Prochilodus lineatus*) and tambaqui (*Colossoma macropomum*) cultured at high (TC) and low (TLC) biomass. Bent – Benthic organisms.

| Functional Group | Biomass (g $m^{-2}$) | P/B (210 $days^{-1}$) | Q/B (210 $days^{-1}$) | Ecotrophic Efficiency (EE) | Effective Trophic Level | Feed Import (g $m^{-2}$ 210 $days^{-1}$) | Biomass accumulation (g $m^{-2}$) |
|---|---|---|---|---|---|---|---|
| ***T*** | | | | | | | |
| Phytoplankton | 2.50 | 284.9 | - | 0.774 | 1.000 | - | - |
| Zooplankton | 5.71 | 57.9 | 193 | 0.430 | 2.000 | - | - |
| Bent | 4.28 | 86.4 | 288 | 0.036 | 2.100 | - | - |
| Tambaqui | 66.0 | 1.982 | 6.04 | 0.833 | 2.083 | - | 91.8 |
| Feed | 2.85 | - | - | 0.801 | 1.000 | 599 | - |
| Detritus | 456.7 | - | - | 0.832 | 1.000 | - | 313.6 |
| ***TC*** | | | | | | | |
| Phytoplankton | 4.47 | 284.9 | - | 0.576 | 1.000 | - | - |
| Zooplankton | 10.7 | 41.2 | 137 | 0.737 | 2.000 | - | - |
| Bent | 8.8 | 85.56 | 285.2 | 0.029 | 2.100 | - | - |
| Curimba | 8.67 | 1.54 | 6.51 | 0.923 | 2.786 | - | 12.3 |
| Tambaqui | 71.0 | 1.979 | 6.04 | 0.855 | 2.126 | - | 100.2 |
| Feed | 3.1 | - | - | 0.954 | 1.000 | 651 | - |
| Detritus | 580.8 | - | - | 0.883 | 1.000 | - | - |
| ***TLC*** | | | | | | | |
| Phytoplankton | 2.10 | 284.9 | - | 0.906 | 1.000 | - | - |
| Zooplankton | 8.21 | 39.5 | 132 | 0.529 | 2.000 | - | - |
| Bent | 2.79 | 84.03 | 280.1 | 0.067 | 2.100 | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Curimba | 40.89 | 0.49 | 3.70 | 0.881 | 2.492 | - | 17.7 |
| Tambaqui | 55.3 | 2.4 | 6.04 | 0.792 | 2.109 | - | 59.9 |
| Feed | 2.08 | - | - | 0.925 | 1.000 | 436 | - |
| Detritus | 409.2 | - | - | 0.913 | 1.000 | - | - |

The total system throughput, the total primary production-to-total respiration ratio, and net system production were all higher in TC, with lower values observed in TLC (Table 5). The total biomass excluding detritus was greater in TLC and lower in T. The average path length was lower in TLC, with similar values in T and TC. The connectance index was similar across treatments. The system omnivory index was higher in TC and similar in T and TLC. Ascendency was higher in T and lower in TC. Finn's cycling index was highest in TC and lowest in TLC. The Ecopath pedigree of the models reached a maximum of 0.555.

Table 5. Summary statistics for the Ecopath models of tambaqui (*Colossoma macropomum*) monoculture (T) and IMTA system integrating curimba (*Prochilodus lineatus*) and tambaqui (*Colossoma macropomum*) cultured at high (TC) and low (TLC) biomass.

| Parameter | T | TC | TLC |
|---|---|---|---|
| Total system throughput (g $m^{-2}$ 210 $days^{-1}$) | 6098 | 9683 | 4690 |
| Net system production (g $m^{-2}$ 210 $days^{-1}$) | -176.4 | -152.8 | -197.5 |
| Total primary production : total respiration ratio | 0.801 | 0.893 | 0.752 |
| Average path length | 6.733 | 6.697 | 5.900 |
| Connectance index | 0.598 | 0.542 | 0.520 |
| System omnivory index | 0.047 | 0.063 | 0.051 |
| Ascendency (%) | 39.7 | 37.6 | 38.7 |
| Finn's cycling index (% of total throughput) | 36.8 | 40.2 | 33.2 |
| Ecopath pedigree | 0.571 | 0.556 | 0.556 |

## Discussion

Stable isotope analysis showed that tambaqui based their diet mostly on commercial feed, with only small inputs from natural food sources. Curimba, on the other hand, fed mainly zooplankton, live or dead, but in TLC they also consumed more feed and tambaqui feces. Dietary overlap between the two species was low in TC but increased to 16% in TLC, as indicated by mixing models and isotopic niche analysis. At the ecosystem level, TC supported greater energy flow, production, and nutrient cycling, whereas TLC showed lower ecological efficiency and cycling capacity.

In the three treatments, water quality parameters remained within the ranges recommended for fish production, as outlined by Boyd et al. (2020b) , suggesting favorable environmental conditions for fish growth. The inclusion of curimba in IMTA systems influenced physical water parameters, such as total suspended solids and turbidity, likely due to the species' bottom-digging behavior, which causes bioturbation (Oliveira Junior et al. 2019). This bioturbation, combined with the high feed inputs and the elevated tambaqui biomass in TC, may have enhanced nutrient availability, leading to a higher phytoplankton biomass, as indicated by increased chlorophyll-*a* levels in this treatment (Felip and Catalan 2000; Kasprzak et al. 2008).

The inclusion of curimba in TC had no impact on tambaqui growth, FCR, or SGR. Similar findings were reported by Franchini et al. (2020) in the integrated production of juvenile tambaqui with curimba. $\delta^{13}C$ and $\delta^{15}N$ values of tambaqui and curimba highlighted that both fish species relied on different food sources, reinforcing their compatibility in IMTA systems (Lecocq et al. 2024). Tambaqui demonstrated a stable and uniform food assimilation pattern across different treatments, confirming that the inclusion of curimba did not affect its resource utilization. Its growth was mainly supported by the ingestion of formulated feed, as observed in previous studies (Lima et al. 2024a, b). $\delta^{13}C$ and $\delta^{15}N$ values of tambaqui were enriched by 0.19 to 0.46‰ and by 2.43 to2.67‰ in $^{13}C$ and $^{15}N$, respectively compared to the supplied formulated feed. Additionally, tambaqui consumed negligible quantities of natural food sources available in aquaculture ponds, such as zooplankton, insects, benthic organisms, and sediment. This pattern revealed by stable isotopes were consistent with stomach content observations (Supplementary data), reinforcing the reliability of our inferences. The smaller standard ellipse areas of tambaqui reflect its lower dietary spectrum compared to curimba, likely due to tambaqui 's strong reliance on formulated feed in aquaculture ponds. Conversely, curimba exhibited a larger ellipse, indicating

greater dietary diversity and a broader utilization of food sources within the pond ecosystem.

Curimba's food utilization was influenced by the biomass of tambaqui. In systems with high tambaqui biomass (TC), curimba's growth was primarily supported by zooplankton, followed by sediment and benthic organisms. In systems with low tambaqui biomass (TLC), we observed an increase in the contribution of formulated feed and tambaqui feces, alongside a decrease in the participation of zooplankton as a food source for curimba, even though zooplankton density in the water did not differ between treatments ($P$ = 0.0566; 430 ± 352, 806 ± 530, and 618 ± 573 individuals $L^{-1}$ in T, TC, and TLC, respectively). These changes led to a diet overlap between both species, characterized by overlapping isotopic niches and an increase in the Ecopath's overlap prey index. The rise in feed utilization by curimba (from 5% to 25%) explained the higher FCR of tambaqui in TLC. The increased feed consumption by curimba may be attributed to better access to this food source because of the low density of tambaqui. In higher density, tambaqui may ingest most of the supplied feed because it swims close to the surface, while curimba occupies the pond's bottom. With a lower tambaqui density, curimba had more opportunities to access the feed. It was observed throughout the experiment that the curimba swims up and takes feed during feeding operations. Thus, changes in fish biomass proportions influence resource utilization by fish, increase diet overlap between the species, and may modify the ecological efficiency of ponds. As highlighted by Thomas et al. (2021) and Lecocq et al. (2024), changes in species compatibility and complementarity due to biological and ecological processes are key challenges in designing IMTA systems. The species proportions in IMTA systems have been poorly studied, and the effect of fish biomass has never been explored. However, our findings indicate that these relationships influence natural food utilization in ponds and should be considered to optimize nutrient use in aquaculture systems.

Zooplankton and/or detritus derived from zooplankton became the primary food source of curimba in fed aquaculture, even though the species continued to access detritus, its regular food sources in natural environments. Stomach content analysis of curimba revealed the presence of both detritus and zooplankton (Supplementary Table S3), supporting the patterns indicated by the isotopic analysis. This shift suggests a preference for food originating from zooplankton, a nutritionally superior food source rich in essential fatty acids and amino acids, over sedimentary organic matter (Kalous et al. 2012; Nahon et al. 2023). When formulated feed is available, curimba also incorporates it into its diet due to its high palatability and superior nutritional quality, similar to that observed by Schultz et al. (2012) for *Cyprinus carpio*. The shift in feeding behavior of curimba in response to

changes in pond conditions and food availability highlights its opportunistic nature, previously observed by Speranza et al. (2016). These changes decreased the ecological efficiency expected in IMTA systems (Chopin et al. 2012; Boyd et al. 2020) by reducing the utilization of alternative food resources by curimba and increasing fish dependence and formulated feed sharing.

The reliance on zooplankton as the primary food source for curimba in aquaculture ponds challenges the expectation that this detritivorous species would primarily consume organic matter from the sediment. This finding suggests that curimba may occupy different trophic roles depending on the availability of food sources. Additionally, since isotopic analyses reflect the assimilated portion of the diet, it is possible that the sediment ingested by curimba contains a large quantity of dead zooplankton and other organic particles that settled from water, and this material was selectively assimilated. Speranza et al. (2016) observed that the fatty acid composition in the feed obtained from the curimba gut was comparable to that from settled material and differed from that of 0-1.5 cm sediments in the river bottom, indicating that curimba fed on the interfacial water/soil layer. Bayo and de Yuan (1996) studied a natural population of *P. lineatus* in Paraná river, South America. These authors concluded that the species eat phytoplankton and zooplankton in the first stages and gradually shift to fresh detritus derived from plankton and periphyton. Regardless of curimba ingests live or dead zooplankton, the species can still be considered an extractive component in IMTA systems, as it depends on natural food resources produced within the system (Troell et al. 2009; Boyd et al. 2020). This scenario demonstrates an indirect beneficial relationship between species through the decomposition of waste materials, which enriches the system with nutrients, supporting phytoplankton and zooplankton production, which is a bottom-up control mechanism, as described by Thomas et al. (2021). This highlights how waste from one compartment serves as an input for others, sustaining the pond's food web. In aquaculture ponds, food web interactions can be reinforced not only through feed input but also by the common practice of fertilization, which further supports primary productivity and nutrient cycling.

In TLC, the lower tambaqui biomass resulted in higher individual tambaqui weights, confirming that growth is reduced by production intensification (Woynárovich and Van Anrooy 2019). However, this scenario also led to a lower FCR for tambaqui, due to increased diet overlap with curimba, as evidenced in the overlap of isotopic niches in this treatment. TC exhibited a higher yield than T with the same feed inputs, indicating an improvement in the nutrient recovery within the pond system. This finding supports the idea that integrating curimba and tambaqui at high biomass promotes ecological intensification and contributes to restorative aquaculture by enhancing ecosystem-level water quality biomitigation, thereby

improving the quality of water discharged into the environment (Aubin et al. 2019; Alleway et al. 2023).

The $\delta^{13}C$ and $\delta^{15}N$ values of formulated feed were distinctly different from those of natural food sources, making them useful for tracing nutrient flows in aquaculture ponds (Nahon et al. 2024). Sediment was $^{13}C$-depleted and $^{15}N$-enriched compared to feed, while it was $^{13}C$- and $^{15}N$-enriched relative to tambaqui feces. These differences indicate that, in addition to waste feed and tambaqui feces, other materials such as dead plankton, insects, and bacteria contribute to sediment composition, as discussed by Yokoyama et al. (2009). Tambaqui feces were more $^{13}C$-depleted than feed but had a similar $^{15}N$ value, likely due to selective absorption of $^{13}C$-enriched nutrients and the less pronounced nitrogen fractionation resulting from efficient protein digestion and assimilation, a pattern also observed in mammals (Hwang et al. 2007; Yokoyama et al. 2009).

While isotopic analysis allowed us to describe the feed assimilated for fish species, Ecopath provided insights into the complex material and energy flow processes within the pond ecosystem. Ensuring mass balance in Ecopath models is a key step in ecosystem modeling (Christensen and Walters 2004). In this study, the Ecopath models were easily balanced. To achieve mass balance, we adjusted the diet composition of zooplankton and benthic organisms, as their diet information was not available from isotopic analysis. Following the rules described by Darwall et al. (2010), balanced models were obtained for all three treatments. This straightforward balancing process may be attributed to the quality of the data used to construct the models, suggesting that the dataset is representative of the aquaculture systems evaluated and that Ecopath is a suitable tool for ecological modeling in aquaculture. The Ecopath pedigree of the systems was similar, ranging from 0.556 to 0.571, a relatively high value given that Ecopath models typically fall within the 0.164 to 0.675 range (Morissette et al. 2006). This suggests that our models are reliable and have a high level of confidence. The trophic levels of tambaqui and curimba calculated by Ecopath (2.11 and 2.64, respectively) were consistent with the second trophic level reported in FishBase for these species (2.02 and 2.18, respectively) (Froese and Pauly 2024), reinforcing the reliability of the models.

The ecotrophic efficiency (EE) describes the proportion of production utilized within the system (Christensen et al. 2024). In our treatments, EE values ranged from 0.036 to 0.954. Benthic organisms exhibited the lowest EE across all treatments, indicating minimal predation by other functional groups in the ponds. This highlights an opportunity to enhance system complexity by introducing a species capable of utilizing this food resource, such as the Amazon River prawn

*Macrobrachium amazonicum*. Both fish species exhibited high EE values, as expected, because they were fully harvested at the end of the production cycle (Xiao et al. 2024). The EE of tambaqui remained consistent across treatments, suggesting a high energy conversion efficiency within the ecosystem (Xiao et al. 2024). In contrast, curimba displayed slightly higher EE values in TC compared to TLC, indicating more efficient energy conversion in TC, where assimilation relied more on natural food sources. The high EE of formulated feed reflects the strong influence of artificial food pellets in sustaining the pond ecosystem, thereby increasing it carrying capacity as observed by Bayle-Sempere et al., (2013) and Gamito et al. (2020). Additionally, the higher EE of feed in IMTA systems compared to monoculture suggests a more efficient utilization of this resource when a co-cultured species is incorporated, promoting better nutrient use (Checa et al. 2024).

The pond systems effectively recycled and reused detritus within the food web, as evidenced by their high EE, similar to observations in other aquaculture production systems (Feng et al. 2018; Dong et al. 2021; Xiao et al. 2024). Phytoplankton exhibited a higher EE in TLC, where the proportionally greater biomass of zooplankton, that is one of its main predators, exerted increased grazing pressure. In contrast, the lower EE of phytoplankton in TC suggests an opportunity to introduce organisms that can feed on this group in this treatment. Additionally, Heymans et al. (2016) indicate that phytoplankton EE is related to the trophic state of the water, approaching 1 in oligotrophic waters and falling below 0.5 in waters experiencing plankton blooms. The EE values observed in our systems align with chlorophyll-a concentrations, with the lowest EE recorded in TC, where chlorophyll-a levels were highest, and the highest EE in TLC, where chlorophyll-a concentrations were lowest. Zooplankton EE was lower in T, where it was grazed solely by tambaqui, but increased in IMTA systems, where both tambaqui and curimba preyed upon this group. Notably, zooplankton EE was higher in TC than in TLC, as curimba consumed 22% more zooplankton in TC as observed in the isotopic mixing models, resulting in greater predation pressure on this functional group. These findings underscore the role of species' interactions in shaping energy flow dynamics and highlight the potential for optimizing resource use in IMTA systems.

The total system throughput represents the overall system size in terms of energy flow. This was highest in TC and lowest in TLC, aligning with the total final fish biomass harvested in each pond. Net system production, defined as the difference between total primary production and total respiration, serves as an indicator of ecosystem maturity. This indicator was negative across all treatments, as has been observed in others fed aquaculture systems (Christensen et al. 2005), reinforcing that aquaculture ponds with high feed supplies are primarily

sustained by formulated feed rather than natural primary production. Similarly, the total primary production-to-respiration (P/R) ratio, another key descriptor of system maturity, was below 1 in all treatments. In mature ecosystems, this ratio approaches 1, indicating a balance between energy fixation and maintenance costs, whereas values below 1 are typically associated with organic pollution. The observed P/R values suggest organic accumulation in the system, likely due to the high input of formulated feed, which is not fully utilized and contributes to organic matter buildup.

The connectance index and system omnivory index provide insights into the complexity of food webs within an ecosystem (Xiao et al. 2024). In this study, the connectance index remained consistent across treatments, while the system omnivory index was slightly higher in TC and lower in T, indicating a more complex food chain in the IMTA system (Christensen et al. 2005; Xu et al. 2011; Gamito et al. 2020). The low system omnivory index observed in our study is characteristic of artificial ecosystems such as aquaculture ponds, which have a simplified trophic structure and a lower diversity of functional groups (Aubin et al. 2021). Despite this, our study reported a higher connectance index compared to other aquaculture systems described by Aubin et al. (2021), Bayle-Sempere et al. (2013), Feng et al. (2018), and Xu et al. (2011), indicating a system with greater interconnections between its functional groups. Beyond these general patterns, the trophic structure of the three pond systems revealed clear differences that help explain their ecological efficiency. In monoculture (T), the food web was relatively simple, dominated by direct transfer from formulated feed to tambaqui, with limited utilization of natural resources. In TC, curimba occupied a complementary niche by relying mainly on zooplankton and detritus, while tambaqui remained dependent on feed. This reduced dietary overlap, increased zooplankton ecotrophic efficiency, and enhanced nutrient cycling, as reflected by higher Finn’s cycling and system omnivory indices. In TLC, however, curimba shifted towards greater use of feed and tambaqui feces, resulting in higher prey overlap and lower ecological efficiency. Taken together, these trophic differences show that maintaining a high tambaqui biomass in IMTA not only sustains total fish yield but also promotes more effective recycling of natural resources, thereby reinforcing the system’s contribution to sustainability.

Aquaculture ecosystems are typically designed with simplified ecosystem structures to maximize yields, making them fragile and susceptible to disruption (Feng et al. 2018). Ascendency quantifies both system activity and the degree of organization in material processing, with higher values indicating increased system size, organization, and resilience (Jørgensen 2002; Christensen et al. 2005). In our study, ascendency values were similar across treatments, suggesting comparable

ecosystem structures and resilience (Feng et al. 2018). Similar ascendency values have been reported in tilapia IMTA systems (Xu et al. 2011) and shrimp-crab polyculture systems (Feng et al. 2018). The Finn Cycling Index (FCI), which measures the proportion of an ecosystem's throughput that is recycled, was highest in TC and lowest in TLC. A higher FCI indicates greater nutrient recycling and stronger system resistance to disturbances (Xiao et al. 2024). Thus, among the analyzed systems, TC demonstrated a stronger capacity for nutrient recycling and a more complex food web. Overall, the findings highlight that integrating curimba and tambaqui with high biomass in aquaculture ponds can enhance nutrient recycling and food web complexity, ultimately improving ecological efficiency without compromising system resilience.

The application of modeling tools, such as stable isotope analysis for dietary reconstruction and Ecopath for ecosystem dynamic modeling, offers valuable insights into the functioning of aquaculture systems. These approaches provide an integrative framework for assessing trophic interactions and resource use efficiency. However, it is essential to acknowledge the methodological limitations inherent to both techniques. In isotopic mixing models, dietary sources were aggregated into broad functional groups (e.g., zooplankton, benthos), which may overlook species-specific feeding selectivity exhibited by fish. Besides, the category nominated as zooplankton contains seston larger than 68 µm, which includes live and particulate matter. This classification is common in former studies, such as da Costa et al. (2024) and Nahon et al. (2024). In addition, stomach content analysis was based on a limited number of specimens. For this reason, detailed stomach content results are presented in the Supplementary data. Nevertheless, the qualitative agreement between stomach contents and isotopic reinforces the robustness of our conclusions. Moreover, stable isotope data reflects assimilated, rather than ingested, material, implying that non-assimilated components of the diet may be captured and egested, impacting the trophic web. In addition, Ecopath simulations rely on empirical and estimated parameters based on previous studies, some of which are characterized by low precision, as detailed in the methodology. Beyond methodological considerations, stable isotope analysis integrates diet over weeks to months, which may obscure dietary shifts associated with fish growth during the study. Additionally, the study was conducted over a single production cycle at one location, where local environmental characteristics may have influenced trophic dynamics. These constraints limit the assessment of interannual variability and the extrapolation of results to other aquaculture contexts. Consequently, the findings and model output should be interpreted as approximations rather than exact representations of ecosystem processes. Despite these constraints, the combined use of isotopic and ecological modeling provides a

robust basis for understanding trophic structure and nutrient flows in aquaculture ponds, supporting the development of more sustainable and efficient production strategies.

## Conclusions

Tambaqui and curimba are compatible species for IMTA systems, as their coexistence does not negatively impact each other's growth. Tambaqui follows a consistent pattern of food utilization in fed aquaculture, whereas curimba demonstrates greater dietary plasticity. Given that tambaqui is the primary species in the system, maintaining its biomass at a higher level than curimba's is crucial to minimizing formulated feed sharing between the species while enhancing nutrient recovery and overall system efficiency. Curimba acts as an extractive species in IMTA aquaculture ponds by feeding primarily on zooplankton (live or dead), thereby indirectly contributing to the reduction of organic matter. An IMTA system with curimba and a high tambaqui biomass promotes better utilization of natural food resources in the ponds. IMTA promoted higher ecotrophic efficiency of both feed and zooplankton, as well as a higher system omnivory and Finn's cycling index. The inclusion of curimba increased the total fish biomass harvested from the ponds without requiring additional feed inputs, thereby enhancing the system's nutrient recovery efficiency. This positions the integration of this species as a promising strategy to improve the circularity of aquaculture systems.

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## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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## Supplementary data

Table S1. Mean $\delta^{13}C$ and $\delta^{15}N$ values (± standard deviation) of fish and potential food sources from tambaqui (*Colossoma macropomum*) monoculture (T) and IMTA system integrating curimba (*Prochilodus lineatus*) and tambaqui (*Colossoma macropomum*) cultured at high (TC) and low (TLC) biomass.

| Fish species and food sources | n** | T | | TC | | TLC | |
|---|---|---|---|---|---|---|---|
| | | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) |
| Tambaqui | 15-20 | -17.70 ± 0.31 | 6.45 ± 0.16 | -17.51 ± 0.36 $^{A}$ | 6.67 ± 0.15$^{*}$ | -17.78 ± 0.55 $^{A}$ | 6.69 ± 0.22 $^{A}$ |
| Curimba | 15-20 | - | - | -20.78 ± 0.64 $^{Bb}$ | 7.15 ± 0.86$^{*}$ | -19.47 ± 0.78 $^{Ba}$ | 7.38 ± 0.64 $^{B}$ |
| Benthos | 3-4 | -20.33 ± 0.39 | 5.19 ± 0.68 | -20.33 ± 1.72 | 5.82 ± 1.17 | -23.33 ± 4.69 | 5.33 ± 1.98 |
| Tambaqui feces | 3-4 | - | - | -19.55 ± 1.62 | 4.70 ± 0.61 | -18.76 ± 1.28 | 4.90 ± 0.67 |
| Sediment | 3-4 | -20.91 ± 1.28$^{*}$ | 3.17 ± 1.54 | -21.07 ± 1.57$^{*}$ | 2.82 ± 1.45 | -20.33 ± 0.80$^{*}$ | 3.11 ± 1.83 |
| Zooplankton | 3-4 | -22.36 ± 2.29 | 6.59 ± 0.87 | -19.95 ± 1.45 | 7.75 ± 2.18 | -21.52 ± 2.23 | 8.05 ± 2.71 |
| Insect | *** | -23.77 | 6.20 | -23.77 | 6.20 | -23.77 | 6.20 |
| Feed | *** | -17.32 | 4.02 | -17.32 | 4.02 | -17.32 | 4.02 |

Lower case letters indicate significant difference between treatments and upper-case letters indicate difference between fish species ($p < 0.05$, Tukey test, $^{*}$Kruskal–Wallis test). ** Number of samples by treatment: The highest value was for TLC, while the lowest values were for T and TC. *** A single sample was used for all treatments.

Table S2. Estimated contributions of food sources to the diets of Tambaqui and Curimba in a Tambaqui monoculture system (T) and IMTA system integrating curimba (*Prochilodus lineatus*) and tambaqui (*Colossoma macropomum*) cultured at high (TC) and low (TLC) biomass.

| | Tambaqui | | | Curimba | |
|---|---|---|---|---|---|
| Food Source | T | TC | TLC | TC | TLC |
| Zoo | 2.6 ± 1.7 | 6.3 ± 10.4 | 5.5 ± 4.7 | 67.9 ± 9.2 | 43.9 ± 10.1 |
| Insects | 2.1 ± 1.3 | 2.0 ± 2.1 | 2.7 ± 1.8 | - | - |
| Feed | 89.5 ± 2.7 | 85.6 ± 14.6 | 86.3 ± 5.1 | 4.6 ± 3.2 | 24.5 ± 11.9 |
| Bent | 3.3 ± 2.4 | 3.9 ± 4.4 | 2.5 ± 1.6 | 9.6 ± 7.3 | 4.8 ± 3.2 |
| Sediment | 2.5 ± 1.7 | 2.2 ± 1.6 | 3.0 ± 2.2 | 10.4 ± 6.6 | 5.3 ± 3.9 |
| Tambaqui Feces | - | - | - | 7.5 ± 5.6 | 21.5 ± 15.0 |

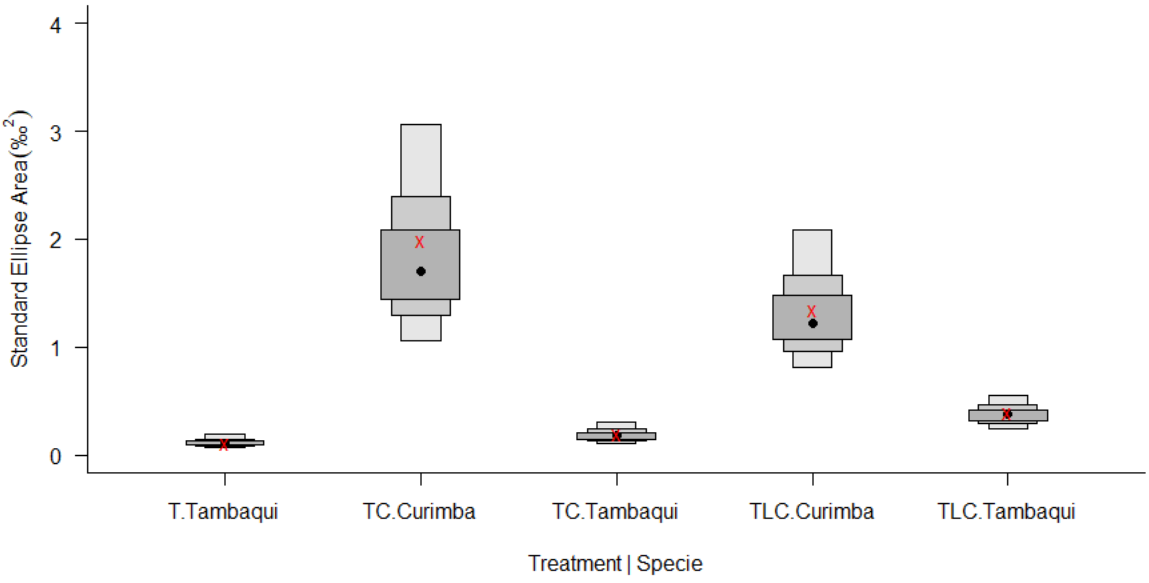


Figure S1. Density plot of the standard ellipse area (SEAc, ‰²) and confidence intervals for Tambaqui produced in monoculture (T) and IMTA system integrating curimba (*Prochilodus lineatus*) and tambaqui (*Colossoma macropomum*) cultured at high (TC) and low (TLC) biomass. Black points represent the mean SEAc, while the shaded gray areas indicate the 95%, 75%, and 50% confidence intervals.

*Stomach content analysis*

In the 5th and 7th months of the experiment, five specimens of each fish species were collected from each pond and euthanized using a eugenol bath (35 mg $L^{-1}$) (Roubach et al., 2005). After euthanasia, stomachs were removed and preserved in 4% buffered formalin until analysis. Stomach contents were examined, and natural food items were classified into the following categories: (a) benthic mollusks; (b) insects – including body parts, larvae, and nymphs; (c) macrophytes and other plants – macrophytes, seeds, and plant tissues; (d) sediment – sand grains; and (e) zooplankton. The frequency of occurrence (FO) of each food item was then calculated as the proportion of stomachs containing the item relative to the total number of stomachs with food (Hyslop, 1980).

Despite the limited number of fish sampled, the analysis revealed the ingestion of the main food items identified in the isotopic analysis for both tambaqui and curimba (Table S3). These patterns were consistent throughout treatments and months, although some variation may reflect the small sample size. Tambaqui exhibited a diversified diet, with insects, zooplankton, and plants being the most frequent food items. Insects were consistently important, whereas zooplankton and plants were more relevant in the sample made in the $5^{th}$ month. In contrast, curimba showed a strong preference for sediment, which was the dominant item across treatments and time. Only minor contributions of zooplankton and macrophytes were detected in the stomach content. Overall, the items recorded in stomach content analysis were similar to those indicated by the isotopic analysis, but with different relative proportions. These differences may reflect food availability, since stomach content represents a snapshot of the recently ingested material, while stable isotopes integrate assimilated resources over weeks to months. Moreover, some food items may be ingested but not assimilated, which could explain discrepancies between the two methods. Finally, the high proportion of sediment in curimba stomachs reinforces the idea that the species ingests not only live zooplankton but also died organisms, assimilating both.

Table S3. Frequency of occurrence of natural food items in the stomachs of tambaqui (*Colossoma macropomum*) and curimba (*Prochilodus lineatus*) from tambaqui monoculture (T) and IMTA systems with curimba and tambaqui at high (TC) and low (TLC) tambaqui biomass.

| Specie | Treatment | Month | Frequency of Occurrence (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Zooplankton | Benthic mollusks | Macrophyte and other plants | Insects | Sediment | n* |
| Tambaqui | T | 5 | 26.7 | 0.0 | 26.7 | 60.0 | 6.7 | 15 |
| | | 7 | 0.0 | 0.0 | 40.0 | 60.0 | 0.0 | 15 |
| | TC | 5 | 53.3 | 0.0 | 46.7 | 20.0 | 0.0 | 15 |
| | | 7 | 33.3 | 0.0 | 20.0 | 46.7 | 13.3 | 15 |
| | TLC | 5 | 30.0 | 0.0 | 25.0 | 15.0 | 0.0 | 20 |
| | | 7 | 0.0 | 15.0 | 30.0 | 35.0 | 0.0 | 20 |
| Curimba | TC | 5 | 7.1 | 0.0 | 0.0 | 0.0 | 78.6 | 14 |
| | | 7 | 33.3 | 0.0 | 0.0 | 0.0 | 86.7 | 15 |
| | TLC | 5 | 5.0 | 0.0 | 10.0 | 0.0 | 75.0 | 20 |
| | | 7 | 0.0 | 0.0 | 0.0 | 0.0 | 55.0 | 20 |

*Number of fish sampled by treatment.

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**Manuscrito 3**

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# Reducing environmental impacts in tambaqui aquaculture: a life cycle assessment of integrated multitrophic aquaculture with curimba

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**Abstract**

This study evaluated the environmental impacts of the integrated multitrophic aquaculture (IMTA) of tambaqui *Colossoma macropomum* and curimba *Prochilodus lineatus* compared to tambaqui monoculture in ponds using life cycle assessment (LCA). The assessment is applied to a pilot-scale experiment conducted in 600 $m^2$ ponds. Collected data consisted of fish biomass, feed and fertilizer amounts, inlet water volume, electricity consumption, and greenhouse gas emissions, complemented by background data from databases and previous studies. The LCA followed a cradle-to-farm gate approach, quantifying nine impact categories for 1 kg of fish biomass produced. IMTA demonstrated reduced impacts across all categories, with substantial decreases in water dependence (38%) and freshwater eutrophication (21%). Additionally, IMTA reduced land occupation by 17%, acidification and cumulative energy demand by 12%, marine eutrophication by 11%, net primary production by 10%, and climate change impact by 9%, compared to monoculture. Feed was the primary contributor to energy demand, net primary production, and climate change, while the rearing step (direct culture emissions) was the main contributor to acidification, water dependency, marine eutrophication, and land occupation. IMTA improved feed conversion ratio and nutrient recovery, key factors driving the reduction in environmental impacts. These results highlight IMTA system as a more sustainable alternative to conventional tambaqui monoculture.

**Key-words:** climate change, *Colossoma macropomum*, integrated culture, freshwater eutrophication, LCA, sustainability, *Prochilodus lineatus*, water dependence

## Introduction

Aquaculture is now the main source of aquatic protein to meet the increasing global demand (FAO, 2025). While some Asian countries are major producers using polycultures and integrated systems such as rice-fish or rice-crustacean farming, modern aquaculture is largely dominated by monoculture practices (Cai et al., 2022). Over recent decades, aquaculture has increasingly shifted toward intensification with the widespread use of pelleted feeds (Boyd et al., 2020). Proposals to boost aquatic food production often focus on species, environments, and advanced technologies that favor high-income markets, prioritizing productivity over resilience through monocultures reliant on anthropogenic inputs (Henriksson et al., 2021). Although these systems are space-efficient, they inherently limit the optimal use of resources, which can lead to significant waste production and nutrient discharge into the environment. Semi-intensive systems, in turn, occupy larger spaces but may offer lower costs, reduced environmental impacts, and greater accessibility for small-scale producers, promoting an inclusive form of aquaculture that balances productivity with conservation (Pomeroy et al., 2014). To address these issues, integrated multitrophic aquaculture (IMTA) systems have been proposed as a strategy to enhance sustainability by improving resource use, including space, and recovering nutrients in the form of farmed biomass (Chopin et al., 2012; Lecocq et al., 2024). IMTA systems enable the combination of different species that utilize different portions of resources or the by-products of one are used by the others, reducing environmental impacts, increasing resilience, and diversifying production (Boyd et al., 2020). Therefore, semi-intensive IMTA systems appear to be a viable alternative for sustainable aquatic food production. However, to fully understand the role of IMTA in promoting sustainability, it is crucial to assess its multi-trait characteristics (Amoussou et al., 2022), especially its environmental implications.

Life cycle assessment (LCA) is a tool used to quantify both local and global environmental impacts of systems and processes (Guinée et al. 2002). It considers all steps (stages) of a product's life cycle, from raw material extraction to the final

disposal of its components (Ghamkhar et al. 2021). In a review, Bohnes et al. (2019) examined 65 studies on the LCA of aquaculture systems and aquafeed production, noting that the farmed species and the feed conversion ratio (FCR) were key factors influencing the environmental performance. Gephart et al. (2021) and Ghamkhar et al. (2021) assessed the impact of aquaculture and found that environmental negative impacts generally decreased with increased intensification. However, they emphasized the need for further research into alternative aquaculture systems that enhance nutrient uptake and production yield per unit of material and energy input, which could help reduce overall emissions - including greenhouse gases and nutrient losses such as nitrogen and phosphorus - per unit of feed, and optimize aquaculture sustainability.

Integrated multitrophic aquaculture systems support production intensification through ecological intensification, which means an increase in production using natural processes and ecosystem services, reducing dependency of industrial supplies (Aubin et al. 2019). IMTA takes advantage of the ecological complementary functions of different species to enhance resource efficiency and sustainability (Aubin et al. 2019; Thomas et al. 2021). This approach involves co-culturing species that occupy different ecological niches, such as fed species alongside organic and/or inorganic extractive species, to produce a diverse range of economically valuable products (Boyd et al. 2020; Lecocq et al. 2024; FAO 2025). These systems improve nutrient recycling by increasing nutrient recovery into biomass, thereby reducing nutrient discharge into the environment. Despite the theoretical potential benefits of IMTA systems, LCA comparisons between monoculture and IMTA systems are limited in aquaculture. Cao et al. (2013) reported improvements in climate change and eutrophication impacts in IMTA systems but found no reductions in acidification or eutrophication when comparing shrimp IMTA to monoculture. Kluts et al. (2012) analyzed intensive striped catfish *Pangasianodon hypophthalmus* monoculture and its integration with rice and other fish species, concluding that monoculture contributed more to climate change and acidification but less to general eutrophication and freshwater eutrophication.

Conversely, Medeiros et al. (2017) found no improvements in environmental impact when comparing tambaqui *Colossoma macropomum* and prawn *Macrobrachium amazonicum* IMTA to tambaqui monoculture during the first grow-out phase. However, when compared to prawn monoculture, IMTA significantly reduced all impact categories, mainly due to the prawn's lower productivity and higher feed conversion ratio relative to fish production. Pouil et al. (2024) observed reductions in most impact categories when comparing the monoculture of *Osphronemus goramy* with its integration with *Azolla filiculoides*. More recently, Hala et al. (2024) reviewed LCA studies on IMTA and found that these systems generally exhibit lower environmental impacts than monoculture while providing economic benefits through nutrient circularity. However, they emphasized the limited number of studies available, highlighting the need for further research on these systems.

Brazil holds the third position in aquaculture production in the Americas and ranks 21st globally (FAO 2025). Nile tilapia *Oreochromis niloticus* is the most produced species, followed by the Amazonian fish tambaqui *Colossoma macropomum* (FAO 2025). Tambaqui production is primarily concentrated in the Amazon region, where cultural and market preferences favor the species. Additionally, regulatory restrictions on non-native species have limited the expansion of Nile tilapia farming in the area (Pacheco et al. 2025). Pacheco et al. (2025) pointed out that aquaculture is one solution to support the sustainable development of the Amazon region and highlighted the need to reduce the environmental impact of aquaculture systems, identifying key challenges such as lowering greenhouse gas emissions, minimizing land-use changes, reducing biodiversity impacts, and decreasing nutrient discharge. Tambaqui is predominantly cultivated in monoculture systems (Valenti et al. 2021). Driving its production to an IMTA system may be a solution to expand aquaculture while mitigating environmental impacts, as reviewed by Hala et al. (2024) for different species. Various models of IMTA systems are described in the literature. In this context, we propose an IMTA system combining tambaqui with curimba *Prochilodus lineatus*, a detritivorous, low-trophic-level species that is among the most abundant and widely

distributed freshwater fish in South American rivers (Sivasundar et al. 2001). This makes curimba an ideal candidate to IMTA, as its detritivorous nature effectively recycles nutrients by consuming organic matter from primary species production. Additionally, curimba farming has a long tradition in Brazil, ensuring the availability of juvenile fish for the aquaculture industry (Valenti et al. 2021).

Some LCA studies have shown that IMTA can reduce environmental impacts compared to monoculture. However, the specific benefits of combining tambaqui, a key species in Brazilian aquaculture, with complementary species like curimba are still largely unknown. Tambaqui production is dominant in the Amazon region, where sustainable aquaculture practices are gaining increasing attention. At the same time, conventional tambaqui monoculture poses environmental challenges. To address this knowledge gap, we applied life cycle assessment to compare the environmental performance of tambaqui monoculture and tambaqui–curimba IMTA system. Our goal is to generate insights into how IMTA systems can improve resource efficiency, reduce environmental impacts, and support the sustainable development of aquaculture in Brazil.

## Methodology

### *Goal and scope*

This study evaluates the environmental relevance of the ecological intensification of tambaqui *Colossoma macropomum* production by integrating it with curimba *Prochilodus lineatus* in an IMTA system at an experimental scale. The experimental data used were obtained from a study designed to compare tambaqui production in ponds in both monoculture and IMTA with curimba. All environmental impacts were calculated per kilogram of live fish produced. In IMTA, tambaqui and curimba were considered a single output. The system boundaries for this assessment were defined as “cradle to farm gate” (Figure 1). The inputs considered for the production stage included feed, fish juveniles, equipment, infrastructure (ponds and buildings), fertilizers, transport, electricity, water (rain and river), and land. Emissions to the air, water, and soil were also taken into account.

Similar inputs and emissions were considered in the design of the earlier stages of fish production, such as eggs, larvae, fry, and juveniles.

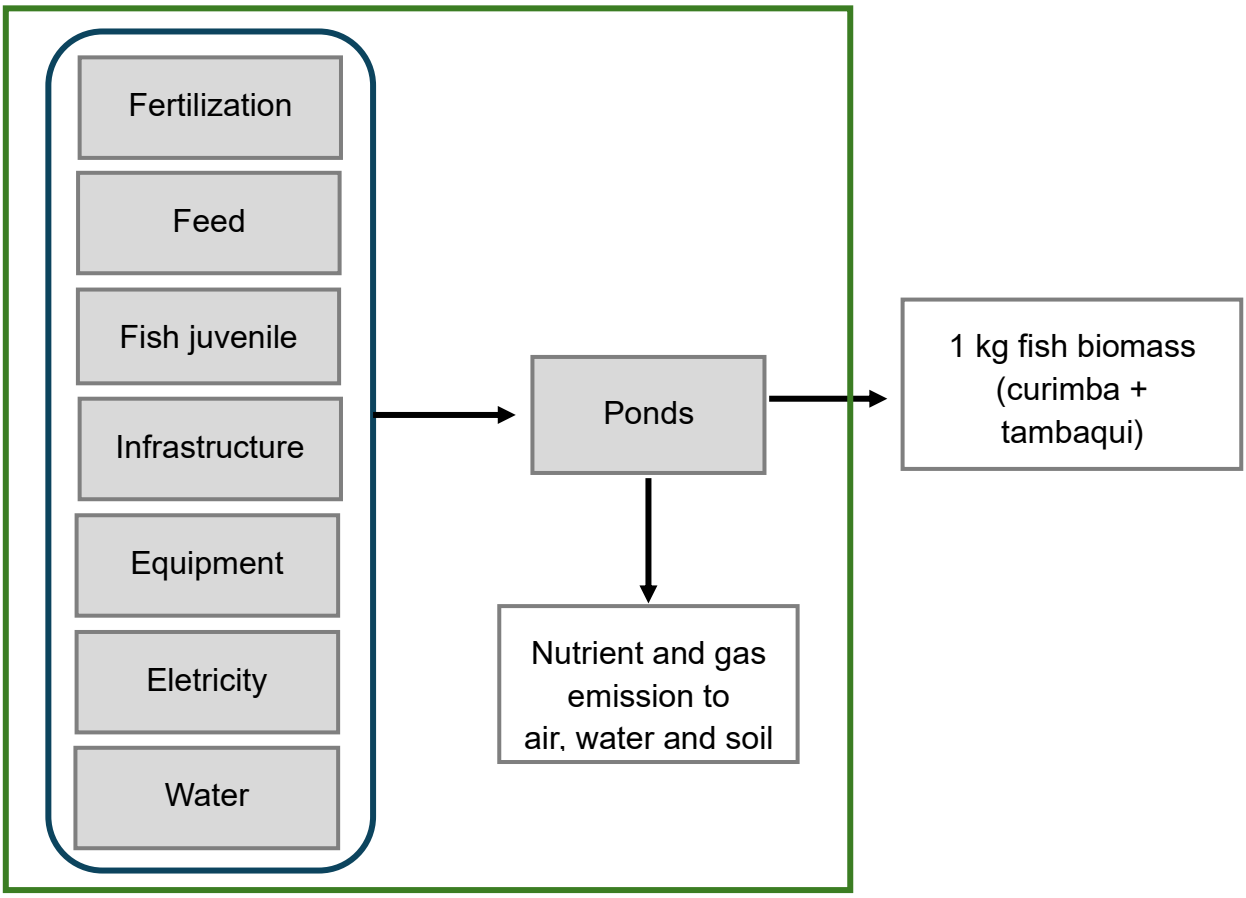


Figure 1. System boundaries for tambaqui *Colossoma macropomum* monoculture and IMTA system of tambaqui and curimba *Prochilodus lineatus.* Inside the blue box are listed the major supplies needed for fish farming. Ponds represent the fish rearing step (stage). Impacts were computed based on the production of 1 kg of fish biomass.

*Main characteristics of each production system*

The study was conducted at the Aquaculture Experimental Center of the Brazilian Agricultural Research Corporation in Palmas, Tocantins, Brazil (10°8'1.33"S, 48°19'9.86"W). A completely randomized design was used, with two treatments and three replicates per treatment. The experiment evaluated tambaqui (stocked with 29 ± 4 g, at 0.4 fish $m^{-2}$) reared either in monoculture or in IMTA with

curimba (stocked with 15 ± 9 g, at 0.3 fish $m^{-2}$). The study was conducted in 600 $m^2$ earthen ponds with a depth of 1.3 m. The experiment lasted seven months, during which tambaqui reached an average weight of 1.7 kg, while curimba reached 0.15 kg. Before the start of the experiment, ponds were drained, disinfected with quicklime (200 g $m^{-2}$), limed with limestone (200 g $m^{-2}$), and fertilized with urea (5 g $m^{-2}$), simple superphosphate (3 g $m^{-2}$), and rice bran (10 g $m^{-2}$). The ponds were subsequently filled with water from a local dam, located less than 1 km from the farm, using gravity flow. Throughout the study, water was added only to compensate for evaporation and seepage. Fertilization was applied during the first three months whenever water transparency exceeded 60 cm. Aeration was provided when dissolved oxygen levels dropped below 3 mg/L, and the total hours of aeration were recorded to calculate electricity demand. The target species was tambaqui, which was fed a commercial extruded feed. Every month, 30 tambaqui were weighed, and their biomass was used to adjust the amount of feed offered. Fish with a weight range of 30 to 230 g, 230 to 410 g, and 410 to 700 g were fed a diet containing 32% crude protein, at feeding rates of 4.5%, 3%, and 2% of body weight per day, respectively. For fish weighing between 700 and 1,130 g, and those exceeding 1,130 g, a feed containing 28% crude protein was provided at feeding rates of 2% and 1.5% of body weight per day, respectively (see Supplementary Data A for more details). Feeding was over when the fish ceased eating or when the daily dose, computed by feed rate, was reached. The quantity of feed offered in each pond was recorded. Data on the rearing performance are presented in Table 1.

Table 1. The mean ± standard deviation of the productive performance of tambaqui *Colossoma macropomum* monoculture (T) and IMTA system of tambaqui and curimba *Prochilodus lineatus* (TC) in ponds. FCR – Feed conversion ratio.

| | T | TC | *P*-value* |
|---|---|---|---|
| *Tambaqui* | | | |
| Survival (%) | 58 ± 14 | 60 ± 14 | 0.8397 |
| Yield (kg/ha) | 3783 ± 383 | 4116 ± 550 | 0.4258 |
| FCR tambaqui | 1.73 ± 0.16 | 1.73 ± 0.25 | 0.9851 |
| *Curimbatá* | | | |
| Survival (%) | - | 97 ± 6 | - |
| Yield (kg/ha) | - | 561 ± 179 | - |
| Total Yield (kg/ha) | 3783 ± 383 | 4683 ± 550 | 0.0831 |
| Total distributed feed (kg/ha) | 6512 ± 377 | 7067 ± 862 | 0.3596 |
| FCR total species | 1.73 ± 0.16 | 1.52 ± 0.23 | 0.2698 |

* *t*-test

*Life cycle inventory*

The inventory data for this study incorporated SimaPro databases, original data collected from the experiment, and information from previous studies. Data from the rearing system obtained from field measurements included final fish biomass, feed and fertilizer amounts, inlet water volume, and electricity consumption. For electricity consumption, only the energy used for aeration was considered, as the water supply to the ponds was gravity-fed. Electricity consumption did not differ significantly between systems ($P$ = 0.9327), with values of 343 ± 347 kWh in the T and 371 ± 262 kWh in TC. The river water input to each pond was the sum of water used to fill the ponds and that required to replace water lost through infiltration and evaporation, minus the input from rainfall. Evaporation and infiltration were measured monthly by monitoring changes in water depth over 96 hours with no added water. Rainwater input was measured daily using a rain

gauge. Water consumption did not differ significantly between systems ($P$ = 0.1464), with values of 4,883 ± 751 m³ in T and 3,894 ± 203 m³ in TC. Similarly, the number of fertilization applications per pond did not differ between treatments ($P$ = 0.8166), with 2.3 ± 0.5 applications in T and 2.3 ± 1.2 in TC.

Local emissions of nutrients associated with fish growth were estimated using nutrient-balance modeling (Cho and Kaushik 1990). The calculation of nitrogen (N) and phosphorus (P) emissions was based on the difference between the nutrients provided to fish through the feed and the fertilizer, and the amount assimilated as fish weight gain. The nutrient digestibility of the feed, fish body composition, and the uneaten portion of the distributed feed were used to calculate the fractions of emitted N and P (see Supplementary Data B for detailed information). The nutrient input from river water was not included in the mass balance, as these nutrients did not originate from fish production activity. Therefore, only the additional nutrients in the water output were considered. The nitrogen and phosphorus content in the commercial diet, fertilizers (simple superphosphate and rice bran), and tambaqui were calculated by multiplying the mean N and P concentrations in the diet, fertilizers, and tambaqui by the total amounts of diet, fertilizer supplied, and fish biomass, respectively. The nitrogen contents were obtained according to AOAC (2005; method 988.05), and the protein contents were calculated by multiplying the nitrogen obtained by 6.25. The phosphorus contents were determined according to the method presented in Embrapa (2009). The crude protein content of the feed was based on the feed label. For curimba, nitrogen content was estimated by assuming a crude protein value of 19% (Maia et al. 1999). The nitrogen input from urea fertilizer was calculated considering that urea contains 45% nitrogen (Boyd and Lichtkoppler 1979).

It was assumed that nitrogen and phosphorus accumulated in the pond bottom accounted for 18.8% of total nitrogen input and 8.8% of total phosphorus input, respectively based on Flickinger et al. (2020) and Flickinger et al. (2019). The emissions of $CH_4$, $N_2$, and $N_2O$ to the atmosphere through ebullition were measured monthly. The average monthly emissions were then used to calculate

the total emissions for the production cycle. The total emissions were then estimated based on the assumption that ebullition accounted for 85% of total methane emissions and 100% of total $N_2$ emissions (Flickinger et al., 2020; Flickinger et al., 2019; Vroom et al., 2023). As there is no information on the proportion of $N_2O$ emitted through ebullition and diffusion in aquaculture ponds, we considered only $N_2O$ emissions from the ebullitive process. $CO_2$ emissions from the pond were not considered in this study, as they originate from biogenic carbon and do not contribute to additional $CO_2$ emissions in the atmosphere (Stichnothe and Hospido 2008). Two fiberglass funnels, suspended by floats, were placed on the surface of the ponds each month to capture gas bubbles. A graduated bottle was connected to the end of each funnel to trap gas bubbles emitted over a 24-hour period. The gas samples were collected in transfer tubes and analyzed by gas chromatography (Shimadzu Instruments GC-2014 Permanent Gas Analyzer, Japan) using TCD (Thermal Conductivity Detector) and FID (Flame Ionization Detector). Average monthly emissions of $N_2$ and $N_2O$ were incorporated into the mass balance calculations, whereas $CH_4$ were considered separately as direct emissions. Ammonia volatilization was considered to account for 12.5% of total nitrogen inputs (Gross et al. 2000). The inputs and outputs used to build the inventories are presented in Supplementary Data C.

Most of the background data were obtained from the ecoinvent® 3 database, except for some feed ingredients (cotton seed, maize grain, soybean meal, and sunflower meal), which were sourced from the Agribalyse database. The distance for road transport was estimated using Google Maps®. It was assumed that each fish feed ingredient was transported to the feed factory from the Brazilian city that produced the largest quantity of it, as suggested by Medeiros et al. (2017). The pelletizing and packing processes were based on the study by Boissy et al. (2011). Additionally, we included the transport of feed, equipment, and materials used in the infrastructure from the cities where they were manufactured to the experimental farm.

Data on tambaqui fry production were obtained from Medeiros et al. (2017), with adjustments to transport data based on the fry production location. Given the similarities between tambaqui and curimba production, the same data were used for curimba fry production, except for reproduction parameters, which were sourced from Brabo et al. (2015). For the juvenile production phase, which precedes grow-out in aquaculture farms, we considered tambaqui (2.0 ± 0.8 g) and curimba (3.2 ± 2.1 g) fry were sourced from commercial hatcheries located in Brejinho de Nazaré, TO, Brazil (48°35′17.93″ S, 11°1′52.95″ W) and Ipueiras, TO, Brazil (48°27′54.274″ S, 10°58′14.88″ W), respectively. The fry was transported by truck to the experimental farm, where they were stocked in ponds similar to those used in the main trial and fed extruded commercial feed until reaching the target size for the study. A methodology similar to that used for grow-out production was applied in this phase, with detailed data provided in Supplementary Data C (Table C2).

*Life cycle impact assessment*

All life cycle impact calculations were performed using SimaPro® 9.5 (PreConsultants, Amersfoort, The Netherlands). Nine impact categories were selected to evaluate the life cycle impacts of tambaqui production in monoculture and in IMTA with curimba, as these are among the most commonly used categories in aquaculture studies (Bohnes et al. 2019; Ghamkhar et al. 2021). The selected categories include:

- acidification (mol $H^+$ eq), which aggregates substances that release hydrogen ions into the ecosystem;
- climate change (kg $CO_2$ eq), which aggregates greenhouse gas emissions;
- cumulative energy demand (CED; MJ), which aggregates the energy used from renewable and non-renewable sources;
- eutrophication (kg $PO_4^{3-}$ eq), aggregating nutrient emissions that lead to oxygen depletion, impacting aquatic and terrestrial environments, and adding the theoretical oxygen demand calculation for solid wastes from fish farms (Aubin et al. 2009). Although the separate evaluation of marine and

freshwater eutrophication is a more recent methodological approach, we also included the eutrophication category to facilitate comparison with previous studies. However, the eutrophication results were not shown in the figures;

- freshwater eutrophication (kg P eq), aggregating substances that release phosphorus into the ecosystem;
- land occupation ($m^2$ yr), representing the area and duration required to produce an output;
- marine eutrophication (kg N eq), aggregating substances that release nitrogen into the ecosystem;
- net primary production used (NPPU, kg C) refers to the use of a net primary producer as a biotic resource in the sense of being unavailable for other purposes, and was calculated as defined by Papatryphon et al. (2004) and Le Féon et al. (2025);
- water dependence ($m^3$), which included the volume of water used in ponds as proposed for aquaculture production systems (Aubin et al. 2009) and the amount of water used in the other fish production phases.

The impact assessment followed the International Reference Life Cycle Data System (ILCD) (European Commission - JRC - IES 2010) for acidification and climate change, the CML IA method (Guinée et al. 2002) for eutrophication potential and land occupation, and the ReCiPe method (ReCiPe 2016) for freshwater and marine eutrophication. Total energy use was calculated using the Cumulative Energy Demand method (Hischier et al. 2010). All calculations were performed using SimaPro® 9.5 (PreConsultants, Amersfoort, The Netherlands).

*Uncertainty and data analysis*

The process for determining uncertainty in the production systems was adapted for both primary and secondary data. For primary data, the standard deviation of three replicates by treatment was used, assuming a normal distribution, assessed using the Shapiro-Wilk test at a significance level of 0.05 in R software (R Core Team 2025). For secondary data, uncertainty was quantified

using the pedigree matrix from Ciroth et al. (2016), and a lognormal distribution was applied. Monte Carlo (MC) analysis was performed using SimaPro® 9.5. To compare systems while considering uncertainty, we ran 1000 interactions in pairs in an MC analysis. This method indicated the number of times one system had higher impacts than the other. We considered that the two systems differed if one had higher impacts in at least 90% of the runs (Medeiros et al. 2017; Pouil et al. 2024).

## Results

The monoculture of tambaqui exhibited a significantly higher environmental impact than the IMTA of tambaqui and curimba in terms of freshwater eutrophication and water dependence (Table 2). The most pronounced difference was observed in water dependence, where producing 1 kg of fish required 18 $m^3$ of water in T, compared to 11 $m^3$ in TC, representing a 38% reduction (Figure 2). Additionally, IMTA led to a 21% reduction in phosphorus release into the environment, resulting in lower impacts on freshwater eutrophication (Figure 2). A tendency toward reductions in other environmental impacts for IMTA was also observed. Specifically, acidification and cumulative energy demand decreased by 12% in IMTA, while climate change impacts dropped by 9%, eutrophication and land occupation by 17%, marine eutrophication by 11%, and net primary production by 10% (Figure 2).

Table 2. Results of the Monte Carlo analysis (n = 1000) applied to the production of 1 kg of fish biomass in tambaqui (*Colossoma macropomum*) monoculture (T) and IMTA system of tambaqui and curimba (*Prochilodus lineatus*) (TC) in ponds. * Systems were considered significantly different if one exhibited higher impact in at least 90% of the 1000 runs.

| Category | System | Mean | Median | SD | CV | Q1(25%) | Q3 (75%) | T > TC* (%) |
|---|---|---|---|---|---|---|---|---|
| Acidification (molc $H^+$ eq $kg^{-1}$) | T | 0.066 | 0.066 | 0.005 | 6.90 | 0.058 | 0.075 | 85 |
| | TC | 0.058 | 0.057 | 0.005 | 9.13 | 0.047 | 0.069 | |
| Cumulative energy demand (MJ $kg^{-1}$) | T | 44.424 | 44.490 | 7.373 | 16.60 | 30.516 | 60.060 | 67.6 |
| | TC | 38.952 | 38.572 | 7.877 | 20.22 | 23.496 | 54.501 | |
| Climate change (kg $CO_2$ eq. $kg^{-1}$) | T | 4.273 | 4.257 | 0.511 | 11.96 | 3.318 | 5.331 | 69.8 |
| | TC | 3.901 | 3.897 | 0.575 | 14.74 | 2.818 | 4.997 | |
| Freshwater eutrophication (kg P eq $kg^{-1}$) | T | 0.019 | 0.019 | 0.001 | 7.09 | 0.017 | 0.022 | **96.2** |
| | TC | 0.015 | 0.015 | 0.002 | 13.40 | 0.011 | 0.019 | |
| Marine Eutrophication (kg N eq $kg^{-1}$) | T | -0.052 | -0.051 | 0.032 | 62.47 | -0.120 | 0.009 | 58.1 |
| | TC | -0.058 | -0.058 | 0.016 | -27.20 | -0.090 | -0.026 | |
| Eutrophication (kg $PO_4^{3-}$ eq. $kg^{-1}$) | T | 0.103 | 0.103 | 0.022 | 20.92 | 0.062 | 0.144 | 80.8 |
| | TC | 0.081 | 0.081 | 0.012 | 14.73 | 0.059 | 0.105 | |
| Land occupation ($m^2$ year $kg^{-1}$) | T | 5.149 | 5.072 | 0.699 | 13.58 | 3.935 | 6.678 | 81.8 |
| | TC | 4.270 | 4.237 | 0.622 | 14.56 | 3.140 | 5.569 | |
| Net primary production use (kg C $kg^{-1}$) | T | 1.420 | 1.421 | 0.155 | 10.91 | 1.118 | 1.721 | 76.3 |
| | TC | 1.272 | 1.267 | 0.134 | 10.56 | 1.012 | 1.545 | |
| Water dependence ($m^3$ $kg^{-1}$) | T | 18.205 | 18.185 | 2.204 | 12.11 | 13.897 | 22.584 | **99.7** |
| | TC | 11.251 | 11.171 | 0.815 | 7.24 | 9.804 | 13.076 | |

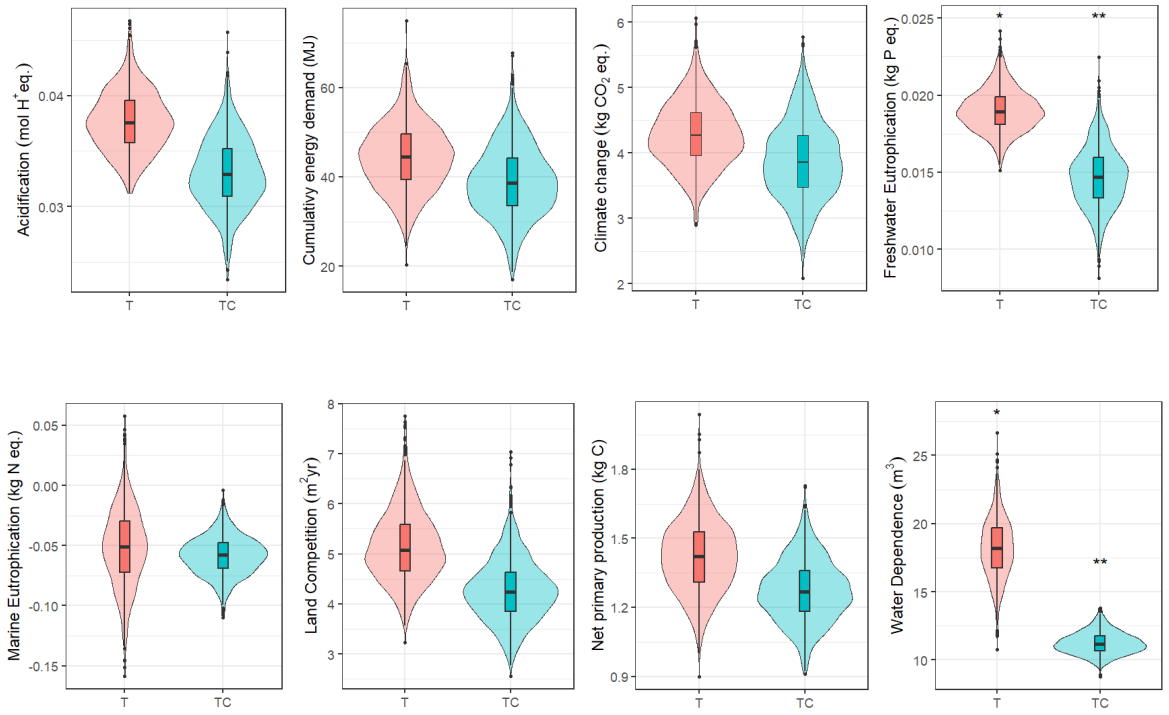


Figure 2. Uncertainties in the environmental impacts per kg of fish of tambaqui *Colossoma macropomum* monoculture (T) and IMTA system of tambaqui and curimba *Prochilodus lineatus* (TC) in ponds. The violin plot combines a boxplot with a kernel density estimate to show data distribution. The width of the shaded area represents the relative frequency of values, with wider sections indicating higher concentration. The central line marks the median, the box shows the interquartile range (IQR), and the whiskers extend to the minimum and maximum values within 1.5 × IQR. Outliers are displayed as individual points. Asterisks denote significant differences between production systems, which were considered significantly different if one exhibited higher impact in at least 90% of the 1000 Monte Carlo simulation runs.

The contributions of rearing system components (rearing, fish juveniles, feed, fertilization, equipment, infrastructure, and electricity) varied depending on both the impact category and the production system (Figure 3). However, the relative ranking of these contributors remained consistent across the two systems. Feed was the dominant contributor to climate change, cumulative energy demand,

and net primary production, accounting for 64–66%, 65–66%, and 88–90% of the impacts, respectively. Electricity was the second-largest contributor to cumulative energy demand (21%), whereas for climate change, the second-largest contributor was the rearing step itself (15–17%). In net primary production, juvenile production accounted for 9–12% of the impact. For all other impact categories, the rearing step was the primary contributor, followed by feed. Specifically, for eutrophication, freshwater eutrophication, and water dependence, the rearing step accounted for over 85% of the impact. In acidification, it contributed 59–60%, while for land competition, it accounted for 57–59%. In the marine eutrophication category, the rearing step showed negative values, outweighing the contributions from feed and other inputs (Supplementary Data D).

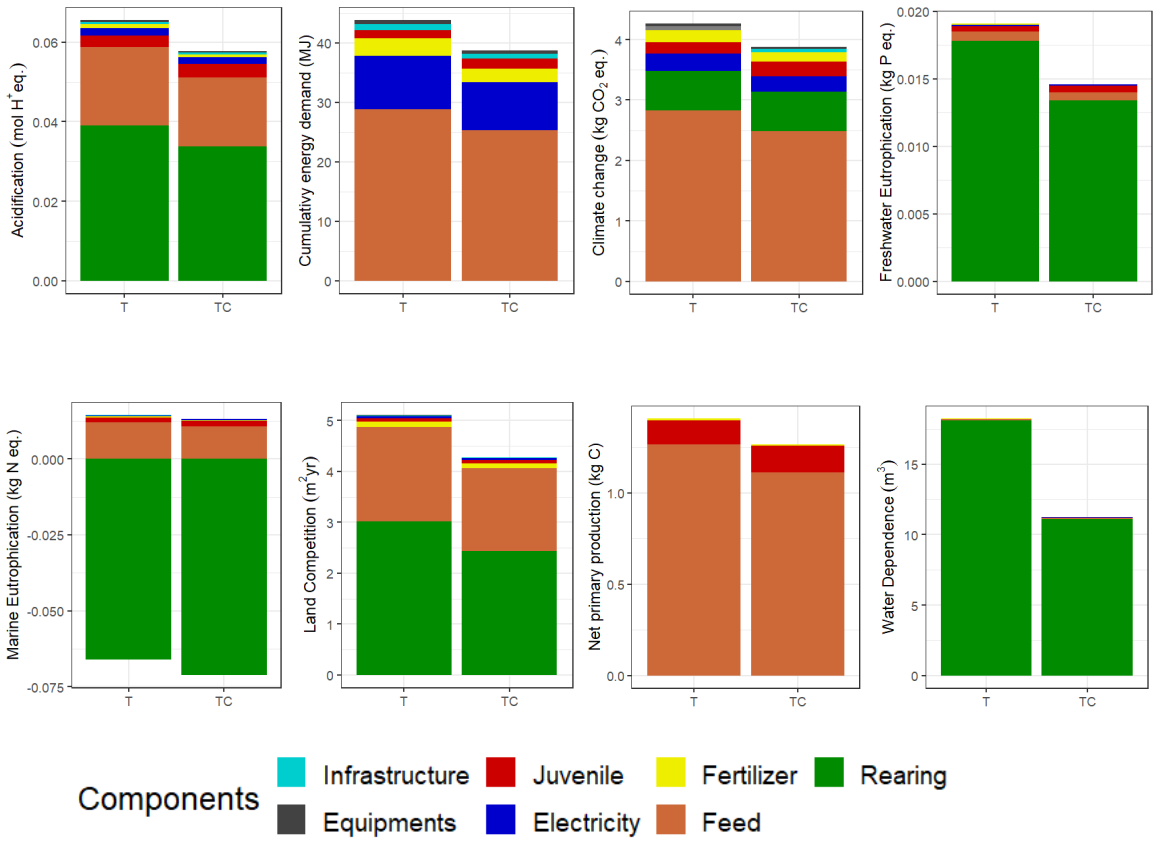


Figure 3. Contribution of each input or production step (rearing) to the environmental impacts of 1 kg of fish biomass in tambaqui *Colossoma*

*macropomum* monoculture (T) and IMTA system of tambaqui and curimba *Prochilodus lineatus* (TC) in ponds.

## Discussion

The IMTA of tambaqui and curimba led to a reduction in all environmental impact categories, with significant decreases observed in freshwater eutrophication and water dependence. Comparative life cycle assessments between IMTA and monoculture systems are still limited in scientific literature. In a review of 13 manuscripts, Hala et al. (2024) assessed life cycle assessments comparing IMTA and monoculture. Their conclusions supports the findings of the present study, i.e., the well-integrated systems, characterized by significant scale and careful species selection, exhibit lower environmental impacts across most categories when compared to conventional monoculture. IMTA enhances nutrient recovery through fish biomass, thereby reducing pollutant discharge into the environment (Lecocq et al. 2024). Bohnes et al. (2019) highlighted that reducing nutrient emissions from fish farming is a key strategy for developing more sustainable aquaculture. Therefore, our results support this perspective and position IMTA as an essential production system for promoting the sustainable development of aquaculture.

The reduction in freshwater eutrophication (~21%), marine eutrophication (~11%) and overall eutrophication (~17%) in IMTA system was primarily associated with the lower contribution of the rearing step to these impact categories, which can be linked to reduced nutrient emissions. A similar reduction (~19%) in eutrophication was observed when the macrophyte *Azolla filiculoides* was integrated to the traditional monoculture of Indonesian giant gourami *Osphronemus goramy*, further emphasizing the enhanced efficiency of the integrated culture (Pouil et al., 2024). The inclusion of curimba in tambaqui culture improved nutrient recovery within the production system, thereby reducing the eutrophication impact of fish farming. As eutrophication is a major concern for farmers and local communities due to the deterioration of water quality (Mungkung et al. 2013), this reduction represents a significant benefit of IMTA systems. These findings align

with the principles of the Integrated Multi-Trophic Aquaculture and the circular economy, which enhance aquaculture sustainability by recycling waste nutrients through the inclusion of low-trophic-level species (Troell et al. 2009; Checa et al. 2024).

The eutrophication values in this study (0.081–0.103 kg $PO_4^{3-}$ eq.) are higher than those reported by Medeiros et al. (2017) for tambaqui monoculture or IMTA of tambaqui *Colossoma macropomum* and Amazon river prawn *Macrobrachium amazonicum* (~0.05 kg $PO_4^{3-}$ eq.). However, Medeiros et al. (2017) only reared fish up to ~180 g, achieving a feed conversion ratio of 1.1, whereas our study covered the entire grow-out phase, with tambaqui reaching a commercial size of 1.7 kg and an FCR of 1.7, even when co-cultured with curimba. These differences in FCR are linked to the fish growth stage, as younger fish typically exhibit higher feed efficiency. This stage difference may explain the higher eutrophication levels observed in our study. On the other hand, the eutrophication impact of tambaqui production, in both monoculture and IMTA, is lower than that of other aquaculture species, such as the low trophic level species Indonesian giant gourami *Osphronemus goramy*, which emits 0.25-0.28 kg $PO_4^{3-}$ eq., likely due to the high use of fertilizers in its production (Pouil et al. 2024). However, tambaqui production shows higher eutrophication values compared to Nile tilapia *Oreochromis niloticus* production in earthen ponds (0.006 kg $PO_4^{3-}$ eq.), according to Yacout et al. (2016), possibly due to the Nile tilapia's better feed conversion ratio of 1.4, as eutrophication is strongly associated with feed input. Ghamkhar et al. (2021) identified the feed conversion ratio as a key factor in mitigating the environmental impact of aquaculture across multiple categories. Thus, systems that generate more biomass from the same amount of feed, such as IMTA, can contribute to mitigating eutrophication. These findings underscore the importance of considering species-specific characteristics, production stages, production systems, and feed management strategies when assessing the eutrophication impact of aquaculture systems.

Most aquaculture studies assess eutrophication potential as an aggregated indicator, rather than distinguishing between freshwater and marine eutrophication (Ghamkhar et al. 2021). However, since phosphorus compounds have a higher eutrophication potential when expressed as $PO_4^{3-}$ equivalents, nitrogen emissions are often underrepresented in these assessments. In the present study, we observed nitrogen uptake from the environment in tambaqui monoculture systems, which was even more pronounced in IMTA system. Flickinger et al. (2019) also reported substantial nitrogen removal through denitrification in tambaqui production in ponds, where nitrogen is released into the atmosphere as $N_2$ gas. In fact, they observed that nitrogen lost to the atmosphere via $N_2$ bubbles exceeded the nitrogen input from formulated feed. Similarly, our study recorded significant nitrogen losses to the atmosphere, resulting in a net negative impact for marine eutrophication. Nitrogen fixation by cyanobacteria was not included in the system boundaries, as it is generally considered negligible in systems with high nitrogen inputs from feed and fertilizers (El Samra and Oláh 1979; Gross et al. 2000). The high nitrogen uptake observed during the fish rearing step (from juvenile to harvest) suggests that the pond system is not only absorbing nitrogen from feed and fertilizers but also utilizing nitrogen already present in the inlet water and accumulated in the sediment. These findings indicate that tambaqui production, whether in monoculture or IMTA, can function as a form of restorative aquaculture by removing excess nitrogen from freshwater systems, particularly in nutrient-rich regions (Alleway et al. 2023). However, in areas with oligotrophic (nutrient-poor) freshwater, supplemental nitrogen inputs may be necessary to support fish growth.

Water consumption in tambaqui monoculture aligned with the average water demand for inland fish production (17 m³) reported by Verdegem and Bosma (2009). In contrast, the IMTA system required less water. The decrease in water dependency in the IMTA was an unexpected result and may be related to changes in pond water and bottom caused by the curimba's digging behavior. This activity led to significantly higher turbidity and suspended solids (data not shown) in the water. The resettling of suspended particles alters the soil particle-size distribution

across the pond bottom, as suggested by Boyd (1995). This process contributes to soil dispersion, which could reduce water loss through infiltration (Yoo and Boyd 1994). Since the ponds were located at the same site, in close proximity, and randomly assigned to experimental units, we hypothesize that soil dispersion—potentially driven by the foraging behavior of curimba—may have contributed to the differences observed between treatments. However, as this process is not yet fully understood, further research is needed to clarify the role of benthic-disturbing species in influencing water consumption in aquaculture ponds.

Feed is the primary input in fed aquaculture, accounting for more than 70% of the effective operational cost in tambaqui production (Belchior and Dalchiavon 2017). It is also the main contributor to aquaculture's impact on cumulative energy demand, climate change, and net primary production, as well as the second-largest contributor to other impact categories evaluated in this study. The significant role of feed in aquaculture's environmental footprint has been widely documented in studies, including polyculture of tiger prawn *Penaeus monodon*, mud crabs *Scylla serrata* and *Scylla olivacea* , milkfish *Chanos chanos*, and Nile tilapia *Oreochromis niloticus* (Aubin et al. 2015), monoculture of Nile tilapia in intensive and semi-intensive ponds (Pelletier and Tyedmers 2010; Yacout et al. 2016), giant gourami *Osphronemus goramy* farming (Pouil et al. 2024), and the review of LCA studies by Ghamkhar et al. (2021) and Hala et al. (2024). NPPU is primarily affected by feed, which represents the use of net primary production as a biotic resource, making it unavailable for other purposes. It reflects the trophic level of the rearing system by measuring the amount of consumed kg C (Papatryphon et al. 2004). Feed is the primary biotic resource in fed aquaculture production and, as such, accounts for 88–90% of this impact, followed by juveniles due to feed consumption during this phase. The NPPU impact of tambaqui production, whether in monoculture or IMTA (~1.35 kg C), is lower than that of other low-trophic species such as Nile tilapia (~11 kg C) and carp *Cyprinus carpio carpio* (~15 kg C) (Mungkung et al. 2013). This difference is mainly attributed to the fact that tambaqui's grow-out phase relies on feed without fish meal or fish oil, ingredients known for their high NPPU impact

(Pelletier and Tyedmers 2010). Therefore, the combination of a low-trophic-level species and a feed free of fish-derived ingredients reduces net primary production use, representing a more sustainable aquaculture strategy.

The improvement in FCR in the IMTA system led to a reduction in NPPU, CED, and climate change impact, aligning with the findings of Ghamkhar et al. (2021). Their review of LCA studies in aquaculture identified a positive correlation between FCR and NPPU and CED. i.e., lower FCR is associated with lower NPPU and CED. On average, 65% of the system's energy demand was attributed by Ghamkhar et al. (2021) to feed processing, with electricity being the second-largest contributor. The contribution of feed and electricity to energy demand varies across studies, depending on the system's reliance on pumps and aerators. In our study, electricity had a lower impact since water was supplied by gravity, and aerators were used for only a short period (an average of 33 out of 210 days of fish production). More than 85% of the energy demand (238 MJ eq kg fish$^{-1}$) in semi-intensive Nile tilapia *O. niloticus* production was attributed to pumps and aerators (Yacout et al. 2016). In contrast, in pond systems without aeration, feed was the primary energy contributor (Pelletier and Tyedmers 2010; Aubin et al. 2015), similar to our findings. These findings highlight that energy demand in aquaculture systems is highly influenced by infrastructure and management practices. When aeration and pumping are minimized, feed becomes the dominant energy contributor, reinforcing the importance of optimizing feed efficiency to reduce environmental impacts.

Feed and rearing steps are the main contributors to land competition, with their impact varying based on the production system. In intensive systems, feed plays a dominant role, while the rearing area has minimal. Conversely, in semi-intensive ponds, rearing contributes significantly to land competition, followed by feed (Ghamkhar et al. 2021). The higher contribution of the rearing step compared to feed in our study is linked to the semi-intensive pond production system. Although it relies on feed, which requires land for ingredient production, it demands

more space due to its lower productivity compared to intensive systems. The reduced land competition in the IMTA system was a result of higher biomass production in the same area and the improved FCR. Thus, IMTA can mitigate land use impacts by enhancing biomass production and feed efficiency. Although plant-based feed, such as the one used in this study, are often expected to increase land competition with other protein sources due to reliance on similar ingredients (Ghamkhar et al. 2021), the present study showed that tambaqui production required less land (4.3–5 $m^2$ kg $biomass^{-1}$) compared to pork (~7.4 $m^2$ kg $biomass^{-1}$), poultry (~6.4 $m^2$ kg $biomass^{-1}$), and beef (23.3 $m^2$ kg $biomass^{-1}$) (Williams et al. 2006) (Figure 4). The land use observed in this study (4.3–5 $m^2$ yr per kg of biomass) was similar to that reported for intensive seabass *Dicentrarchus labrax* farming, which occupies around 4.5 $m^2$ yr per kg of biomass (Jerbi et al., 2012). In contrast, recirculating aquaculture systems (RAS), which achieve a productivity approximately eight times higher than that observed in this study (around 32 t $ha^{-1}$ compared to 4–5 t $ha^{-1}$ here), require roughly six times less land per kg of protein produced (0.7 $m^2$ yr per kg of biomass). On the other hand, extensive fish farming systems, with a productivity more than ten times lower (0.28–0.36 t $ha^{-1}$), show considerably higher land occupation, ranging from 31 to 57 $m^2$ yr per kg of biomass (Wilfart et al. 2013). These findings indicate that plant-based feed did not increase land competition in tambaqui production, and that the production system itself plays a more critical role in impact on land use.

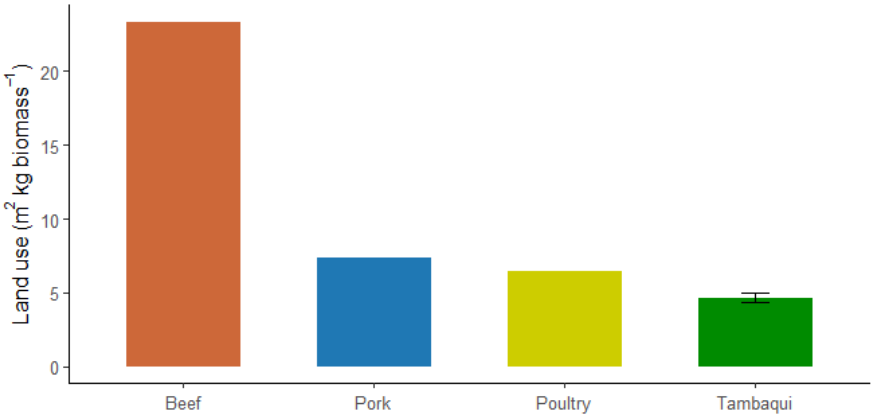


Figure 4. Land-use requirements (m² kg biomass⁻¹) for tambaqui production compared with poultry, pork, and beef. Data for terrestrial livestock were obtained from Williams et al. (2006).

Acidification was primarily driven by the rearing step, which contributed approximately 60% to this impact category. Similarly, Pouil et al. (2024) reported a significant contribution from fish farm operations, for acidification impacts. This is likely associated with nutrient transformations within the pond environment, including the decomposition of fish waste, fragmented feed, and dead fish, resulting in the direct release of acidification compounds into the atmosphere (Hala et al. 2024). Hala et al. (2024), in their review of IMTA production systems, also identified the rearing step as the main contributor to acidification. A 12% reduction in acidification observed in IMTA highlights its potential to mitigate aquaculture impacts through more efficient nutrient utilization. Moreover, improvements in feed conversion ratio and nutrient recovery can significantly reduce nutrient emissions, thereby addressing key drivers of acidification in aquaculture systems.

Climate change reflects the emission of greenhouse gases and is one of the most studied impact categories in life cycle assessments of aquaculture production (Ghamkhar et al. 2021). In a review on LCA in aquaculture, Cao et al. (2013) reported climate change impacts ranging from 2.0 to 10.3 kg $CO_2$ eq. per kilogram of fish produced across different species, production systems, and

locations. The authors also found that IMTA systems perform better in terms of climate change impact compared to monoculture systems, which aligns with our findings, where IMTA reduced climate change impact by 9% (from 4.3 to 3.9 kg $CO_2$ eq. $kg^{-1}$ biomass). A similar reduction was observed by Pouil et al. (2024) in an integrated system with *Osphronemus goramy* and *Azolla filiculoides*. When evaluating the monoculture of sea bream or sea bass and their IMTA with oysters, Beltran et al. (2018) also found a reduction in climate change impact in the IMTA by 1%. The climate change impact of tambaqui production in our study was approximately 50% higher than that reported by Medeiros et al. (2017) for tambaqui monoculture. This difference is likely attributable to the lower feed conversion ratio reported in their study, as the fish were harvested at an earlier growth stage and the $N_2O$ emissions during the rearing period were not considered. Since feed is the main contributor to the climate change impact, the lower FCR in Medeiros et al. (2017) resulted in lower emissions. Similarly, the reduction in climate change impact observed in IMTA in comparison to monoculture in our study (9%) was associated with an improved FCR of 12%, which reduced the use of formulated feed. These results are consistent with those of Pouil et al. (2024), who reported a 10% reduction in climate change impact and a 25% improvement in FCR. The significant role of feed in the climate change impact of aquaculture production has also been reported in other studies (Pelletier and Tyedmers 2010; Yacout et al. 2016; Medeiros et al. 2017; Pouil et al. 2024). These results reinforce the potential of IMTA system improve feed efficiency and mitigate the climate change impact of aquaculture.

Despite feed being highlighted as the main driver of environmental impacts in aquaculture, the rearing step also played a significant role in most of the impact categories evaluated in the present study. Bohnes et al. (2019) identified the rearing step as a key contributor only to eutrophication and water dependency. However, in our study, it was also a major contributor to land competition and acidification. The higher contribution of the rearing step to land competition and water dependency is consistent with the characteristics of pond systems, which

tend to have greater impacts in these categories compared to more intensive systems, such as RAS (Ghamkhar et al. 2021). Consistent with the findings of Bohnes et al. (2019), our results indicate that feed typically impacts more energy demand, net primary production use, and climate change. This discrepancy may be attributed to the IMTA system, which promotes more efficient nutrient utilization. The higher contribution of the rearing step to acidification in our study aligns with the observations of Hala et al. (2024), who evaluated only IMTA systems. Additionally, the use of feed with no fish-derived ingredients during the grow-out phase also contributed to reducing the acidification potential associated with feed, as stated by Papatryphon et al. (2004). These findings underscore the importance of considering the specific characteristics of production systems when evaluating environmental impacts, particularly in life cycle assessments, as they can significantly influence the relative contributions of each production stage.

## Conclusion

The IMTA of tambaqui and curimba reduced all environmental impact categories, with notable decreases in freshwater eutrophication and water dependency. Feed was the main contributor to energy demand, climate change, and net primary production, while the rearing step played a major role in acidification, eutrophication, marine eutrophication, land competition, and water dependency. The higher fish biomass produced in IMTA system improved both the feed conversion ratio and nutrient recovery from the ponds, which were the key factors driving the reduction in environmental impact compared to tambaqui monoculture. Thus, the improved feed efficiency and enhanced nutrient management within the IMTA system were central to its superior environmental performance. These results highlight IMTA with suitable selected species as a more sustainable alternative to conventional monoculture systems in aquaculture. However, since the specific factors responsible for improvements in both feed conversion ratio and nutrient recovery are not yet fully understood, further research is needed to better understand the interactions between species. Additionally, the development of tools and strategies to design efficient IMTA systems is essential,

particularly related to the number and proportion of species and stocking densities. Brazil is a megadiverse country, offering significant potential for the use of different arrays of species, contributing to advancing the integrated aquaculture.

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## Supplementary data

*Supplementary data A*

The feed used in the experiment was a commercial formulated feed produced by Archer Daniels Midland Company (ADM, Brazil). As we don't have access to the relative quantities of each ingredient, we modeled the ingredient assemblage based on the chemical composition of the ingredients, using the ingredients informed in the fed label and based on the data informed by Medeiros et al. (2017) in tambaqui monoculture.

Table A.1 Formulated feed used in the experiment. Ingredient and composition values were based on the feed label. The relative quantities of each ingredient were adapted to achieve the label composition.

| Ingredients | Composition (%) | |
|---|---|---|
| | Fish feed 1 | Fish feed 2 |
| Moisture (max.) | 12 | 13 |
| Crude protein (min.) | 32 | 28 |
| Lipids (min.) | 5 | 4 |
| Ash (max.) | 14 | 10 |
| Fiber (max.) | 5 | 10 |
| Phosphorus (min.) | 0.6 | 0.5 |
| *Feed ingredients* | | |
| Soybean meal | 26 | 24 |
| Sorghum meal | 19 | 15 |
| Wheat bran | 15 | 16 |
| Viscera meal | 15 | 14 |
| Rice bran | 8 | 8 |
| Soybean protein concentrate | 6 | 3 |
| Maize | 4 | 14 |
| Maize starch | 3 | 4 |
| Sunflower meal | 1 | 0 |
| Soybean oil | 1 | 1 |
| Calcitic limestone | 1 | 0 |
| Premix (vitamins and minerals) | 1 | 1 |

*Supplementary data B*

The following assumptions were made to calculate the mass balance of the systems:

(a) N concentration in protein by mass: 0.16.

(b) Uneaten feed in the pond system: 5% of total feed.

(c) Protein and phosphorus digestibility were considered only for tambaqui (*Colossoma macropomum*), as formulated feed was its primary food source. Digestibility values of 90% for protein and 60% for phosphorus were applied, based on data reported by Schneider et al., (2004) for *Oreochromis niloticus*. These values were used in the mass balance calculations.

(d) The nutrient composition of the fertilizers was as follows: urea (45% N, 0.003% P), simple superphosphate (0.54% N, 14.84% P), and rice bran (2.26% N, 2.58% P);

(e) The nutrient composition of the fish was as follows: tambaqui (4% N, 1.87% P), and curimba (3% N, 1.73 P);

(f) The feed conversion ratio (FCR) was calculated considering the final biomass of both species in polyculture.

Equations:

Eq. (B.1) Total N and P input = (Amount feed × N and P in feed) + (Amount fertilizer × N and P in fertilizer);

Eq. (B.2) N feed = Amount of feed × Crude protein (%) in feed × N concentration in protein;

Eq. (B.3) P feed = Amount of feed × P content (%) in feed;

Eq. (B.4) N and P uneaten = N and P feed × % uneaten feed;

Eq. (B.5) N and P waste = Total solid N and P feed waste + Total dissolved N and P feed waste + N and P from fertilizer;

Eq. (B.6) Total solid N and P waste = N and P feed waste + N and P in feces;

Eq. (B.7) N and P feed waste = 5% of N feed;

Eq. (B.8) N feces = N feed × (100% – Protein digestibility);

Eq. (B.9) P feces = P feed × (100% – Phosphorus digestibility);

Eq. (B.10) Total dissolved N and P waste = Digestible N and P – N and P gain;

Eq. (B.11) N and P from fertilizer = (% N and P in fertilizer) × (Quantity of fertilizer applied);

Eq. (B.12) N and P gain = (N and P content in tambaqui × (Feed amount / FCR) × percentage of tambaqui in total biomass) + (N and P content in curimba × (Feed amount / FCR) × percentage of curimba in total biomass);

Eq. (B.13) N emissions to air = $NH_3^+ + N_2 + N_2O$;

Eq. (B.14) $NH_3^+$ volatilization = Total N inputs (fed and fertilization) × % ammonia volatilization;

Eq. (B.15) N emission in the soil = 18.8 % of total N input;

Eq. (B.16) N emission in the water = Total dissolved N waste - $NH_3^+ + N_2 + N_2O$;

Eq. (B.17) P emission in the soil = 8.8 % of total P input;

Eq. (B.18) P emission in the water = Total P waste;

Note: N = nitrogen; P = phosphorus.

*Supplementary data C*

Table C1. Main inputs and outputs used to build the LCA inventory to tambaqui *Colossoma macropomum* monoculture (T) and IMTA of tambaqui and curimba *Prochilodus lineatus* (TC).

| | T | TC |
|---|---|---|
| *Inputs* | | |
| Total Feed (kg) | 390.7 ± 22.6 | 424.4 ± 51.7 |
| Feed 28% (kg) | 228.6 ± 55.4 | 248.0 ± 56.4 |
| Feed 32% (kg) | 162.1 ± 62.9 | 176.5 ± 68.8 |
| Number of Fertilizations[1] | 2.3 ± 0.5 | 2.3 ± 1.2 |
| Tambaqui juveniles (kg) | 11,5 | 11,5 |
| Curimba Juveniles (kg) | - | 4.18 |
| Electricity consumption (khw) | 343 ± 347 | 371 ± 262 |
| River water ($m^3$) | 4117 ± 751 | 3128 ± 203 |
| Rain water ($m^3$) | 766 | 766 |
| Land ocuppation[2] ($m^2$ yr) | 686 | 686 |
| Infrastructure[3] (set) | 1 | 1 |
| Equipment[4] (set) | 1 | 1 |
| *Outputs* | | |
| Final biomass (kg $pond^{-1}$) | 227.3 ± 23.3 | 281.3 ± 33.3 |
| *Emissions to air* | | |
| Methane (kg) | 5.8 ± 3.8 | 7.3 ± 3.8 |
| $N_2$ (kg) | 27.3 ± 14.0 | 31.8 ± 7.0 |
| $N_2O$ ($10^{-5}$ kg) | 7 ± 3 | 5 ± 1 |
| Ammonia | 2.9 ± 0.2 | 3.2 ± 0.5 |
| *Emissions to water* | | |
| Nitrogen (kg) | -15.3 ± 14.3 | -20.3 ± 8.7 |
| Phosphorus (kg) | 3.4 ± 0.4 | 3.1 ± 1.0 |
| *Emissions to soil* | | |
| Nitrogen (kg) | 4.4 ± 0.3 | 4.7 ± 0.7 |
| Phosphorus (kg) | 0.64 ± 0.04 | 0.68 ± 0.10 |

[1] Number of fertilization events: in each event, 1.49 kg of nitrogen and 0.42 kg of phosphorus were applied.

[2] To calculate land occupation, the following areas were summed: (a) the pond area; (b) an area of 80% of the pond area to account for fish pond slopes; and (c) 16% of the pond area for buildings and other uses.

[3] The infrastructure considered included the pond, outlet channels, inlet polyvinyl pipes, and a feed storage building (25 m² for every 30 ponds).

[4] The equipment considered included aerators, nylon ropes, wooden sticks, plastic buckets (1 buckets by each 10 ponds), nylon harvest nets (1 net by each 30 ponds), and a multiparameter sonde (1 sonde by each 30 ponds).

Table C2. Main inputs and outputs used to build the LCA inventory for the production of tambaqui (*Colossoma macropomum*) and curimba (*Prochilodus lineatus*) juveniles.

| | Curimba | Tambaqui |
|---|---|---|
| *Inputs* | | |
| Feed (kg)[1] | 137 | 268 |
| Fertilizers[2] | 1 | 1 |
| Tambaqui fry (und) | - | 4800 |
| Curimba fry (und) | 4800 | - |
| River water ($m^3$) | 158 | 158 |
| Land ocuppation[3] ($m^2$ yr) | 6.53 | 6.53 |
| Infraestructure[4] | 1 | 1 |
| Equipment[5] | 1 | 1 |
| *Outputs* | | |
| Final biomass (kg $pond^{-1}$) | 84.5 | 184 |
| *Emissions to air* | | |
| Methane (kg)[6] | 4.82 | 4.82 |
| $N_2$ (kg)[7] | 1.30 | 2.55 |
| $N_2O$ ($10^{-5}$ kg)[8] | 0.33 | 0.33 |
| Ammonia[9] | 1.15 | 2.06 |
| *Emissions to water* | | |
| Nitrogen (kg)[10] | 2.60 | 3.68 |
| Phosphorus (kg) [10] | 0.64 | 0.85 |

| | | |
|---|---|---|
| *Emissions to soil* | | |
| Nitrogen (kg)[11] | 1.71 | 3.07 |
| Phosphorus (kg)[12] | 0.22 | 0.39 |

[1] Fish feed with 35% crude protein, described in Medeiros et al. (2017).

[2] Number of fertilization events: in each event, 1.49 kg of nitrogen and 0.42 kg of phosphorus were applied.

[3] To calculate land occupation, the following areas were summed: (a) the pond area; (b) an area of 80% of the pond area to account for fish pond slopes; and (c) 16% of the pond area for buildings and other uses.

[4] The infrastructure considered included the pond, outlet channels, inlet polyvinyl pipes, and a feed storage building (25 m² for every 30 ponds).

[5] The equipment considered included nylon anti-bird net, nylon ropes, wooden sticks, plastic buckets (1 buckets by each 10 ponds), nylon harvest nets (1 net by each 30 ponds), and a multiparameter sonde (1 sonde by each 30 ponds).

[6] The methane emission was considered to be 134 mg m² per day, based on Vroom et al. (2023).

[7] The $N_2$ emission was considered to be 20% of total nitrogen input (by feed and fertilizer), based on (Gross et al. 2000).

[8] The $N_2O$ emission was considered to be 1.69 g per kg of fish produced, based on Hu et al. (2012).

[9] The ammonia emission was considered to be 12.5% of total nitrogen input (by feed and fertilizer), based on Gross et al. (2000).

[10] From the mass balance using a similar approach described in the manuscript.

[11] The nitrogen emission to soil was considered to be 18.8% of total nitrogen input (by feed and fertilizer), based on Flickinger et al. (2019).

[12] The phosphorus emission to soil was considered to be 8.8% of total phosphorous input (by feed and fertilizer), based on Flickinger et al. (2020).

*Supplementary data D*

Table D1. Contribution (%) of each input or production step in environmental impacts of tambaqui (*Colossoma macropomum*) monoculture (T) and integrated culture of tambaqui and curimba (*Prochilodus lineatus*) (TC) in ponds.

| Category | | Contribution (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | System | Feed | Rearing | Juvenile | Electricity | Infraestructure | Equipments | Fertilization |
| Acidification (molc $H^+$ eq) | T | 30 | 60 | 4 | 3 | 1 | 1 | 2 |
| | TC | 30 | 59 | 6 | 3 | 1 | 1 | 1 |
| Cumulative energy demand (MJ) | T | 66 | 0 | 3 | 21 | 2 | 1 | 6 |
| | TC | 65 | 0 | 4 | 21 | 2 | 1 | 6 |
| Climate change (kg $CO_2$ eq.) | T | 67 | 15 | 4 | 7 | 2 | 1 | 5 |
| | TC | 64 | 17 | 4 | 7 | 1 | 1 | 4 |
| Freshwater eutrophication (kg P eq) | T | 4 | 93 | 2 | 0 | 0 | 0 | 0 |
| | TC | 4 | 92 | 3 | 0 | 0 | 0 | 0 |
| Marine Eutrophication (kg N eq $kg^{-1}$) | T | 15 | 82 | 2 | 0 | 0 | 0 | 0 |
| | TC | 13 | 84 | 2 | 0 | 0 | 0 | 0 |
| Eutrophication (kg $PO_4^{3-}$ eq.) | T | 9 | 87 | 3 | 0 | 0 | 0 | 0 |
| | TC | 10 | 85 | 4 | 0 | 0 | 0 | 0 |
| Land occupation ($m^2$ year) | T | 36 | 59 | 1 | 1 | 0 | 0 | 2 |
| | TC | 38 | 57 | 2 | 1 | 0 | 0 | 2 |
| Net primary production use (kg C) | T | 90 | 0 | 9 | 0 | 0 | 0 | 1 |
| | TC | 88 | 0 | 12 | 0 | 0 | 0 | 1 |
| Water dependence ($m^3$) | T | 1 | 99 | 0 | 0 | 0 | 0 | 0 |
| | TC | 1 | 99 | 0 | 0 | 0 | 0 | 0 |

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**Manuscrito 4**

Ainda não foi definida a revista para a qual esse artigo será submetido.

# Greenhouse gas emissions in tropical fishponds: diffusive and ebullitive pathways in tambaqui monoculture and integrated system with curimba

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## Abstract

Integrated aquaculture systems have been proposed to improve resource-use efficiency and environmental sustainability, yet their effects on greenhouse gas dynamics in tropical ponds remain poorly quantified. This study evaluated diffusive and ebullitive fluxes of $CO_2$, $CH_4$, $N_2O$, and $N_2$ in tambaqui (*Colossoma macropomum*) monoculture and in an integrated system with curimba (*Prochilodus lineatus*) over a seven-month production cycle in ponds. Gas fluxes were measured monthly using floating chambers and inverted funnels, together with water and sediment monitoring. Monoculture ponds acted predominantly as net $CO_2$ sinks (−1039 ± 2409 mg $m^{-2}$ $day^{-1}$), whereas integrated ponds functioned as net $CO_2$ sources (320 ± 2206 mg $m^{-2}$ $day^{-1}$). Total $CH_4$ emissions ranged from 41.9 ± 64.4 to 55.9 ± 50.2 mg $m^{-2}$ $day^{-1}$, with ebullition contributing about 58% of total methane release and increasing over the production cycle. $N_2O$ fluxes remained near zero in both systems, with no treatment or temporal effects. $N_2$ fluxes showed significant treatment–time interactions, driven primarily by the diffusive pathway. Emissions of $CO_2$, $N_2$, and $N_2O$ occurred almost exclusively through diffusion (>97%), whereas ebullition was relevant only for $CH_4$. Greenhouse gas fluxes were strongly modulated by water and sediment characteristics, with methane ebullition linked to eutrophication indicators and $CO_2$ fluxes governed mainly by inorganic carbon and alkalinity. Integrating curimba into tambaqui ponds did not significantly alter greenhouse gas emission patterns, indicating that productivity gains can be achieved without increasing gaseous emissions in tropical aquaculture systems.

**Keywords:** Fishponds greenhouse gas emissions, diffusive gas fluxes, integrated fish culture, methane ebullition, small water bodies.

## Introduction

Fish is the most widely consumed source of animal protein in the world, and aquaculture is responsible for most of its production, which has steadily increased over the years (FAO, 2024). This growing production, together with rising concerns about the sustainability of food systems, underscores the importance of understanding the potential environmental impacts of aquaculture, particularly its contribution to global warming. Life cycle assessment studies often lack accurate quantification of direct greenhouse gas (GHG) emissions from fishponds, relying instead on estimates (Aubin et al., 2015, 2009; Jerbi et al., 2012; Mungkung et al., 2013). Similarly, Barbosa et al. (2024), in a review of 57 studies addressing nutrient balance in aquaculture systems, reported that only two studies evaluated $CH_4$ and $CO_2$ emissions. Research on direct GHG emissions from aquaculture is relatively recent, and the limited number of published studies has resulted in scarce information on their emission dynamics. Moreover, some studies focus on specific gases, such as carbon dioxide ($CO_2$) (Chen et al., 2016), methane ($CH_4$) (Davidson et al., 2018; Silva et al., 2018; Vroom et al., 2023), or nitrous oxide ($N_2O$) (Gao et al., 2013; Singh et al., 2005; Webb et al., 2019; Xiao et al., 2023), while others investigate particular emission pathways, such as diffusive or ebullitive fluxes (Fang et al., 2022; Waldemer and Koschorreck, 2023). Comprehensive studies that simultaneously quantify all major GHGs are rare (Barreto et al., 2024; Malyan et al., 2022; Yang et al., 2018; Yuan et al., 2019), and, to our knowledge, none have assessed direct $N_2$ emissions together with other gases. Comprehensive assessments of all major greenhouse gases improve the understanding of emission dynamics in aquaculture ponds. However, most existing studies are restricted to temperate regions and rarely quantify both diffusive and ebullitive fluxes.

Methane and nitrous oxide are the most studied greenhouse gases in aquatic systems due to their high global warming potentials (27.2 for methane and 273 for nitrous oxide; IPCC, 2021). Methane is often highlighted as the dominant greenhouse gas in aquaculture systems, whereas $N_2O$ emissions from aquaculture are projected to contribute 5.72% of global anthropogenic $N_2O$ emissions by 2030 (Yuan et al., 2019). In contrast, $CO_2$ emissions in aquaculture ponds largely represent the return of recently fixed carbon to the atmosphere and are therefore commonly not considered a major contributor to the net climate impact of these systems (Biermann and Geist, 2019; Hischier et al., 2010; Stichnothe and Hospido, 2008). Although $N_2$ is not a greenhouse gas, it plays a central role in nitrogen cycling and is tightly coupled to $N_2O$ production and consumption pathways. Consequently, its measurement is essential for evaluating the effects of

anthropogenic nitrogen enrichment on gaseous nitrogen losses (Higgins et al., 2008).

Freshwater ecosystems are important sources of greenhouse gases, and increasing eutrophication strongly influences their emission patterns. Low dissolved oxygen, high organic matter and nutrient concentrations, dominance of primary producers, and frequent algal blooms in eutrophic waters favor greenhouse gas production and release (Li et al., 2021). Eutrophication and greenhouse gas emissions further reinforce each other through feedback mechanisms involving both abiotic and biotic processes (Li et al., 2021). According to Malyan et al. (2022), $CH_4$ emissions from ponds are positively associated with water temperature, total phosphorus, and dissolved organic matter, and negatively associated with dissolved oxygen and sulfate. $CO_2$ emissions correlate positively with nitrogen, phosphorus, and dissolved organic matter, and negatively with pH and dissolved oxygen. In contrast, $N_2O$ emissions increase with nitrate concentration and temperature, with eutrophication acting as a central driver by enhancing labile carbon availability in sediments and reducing oxygen levels in the water column. Therefore, understanding the relationships between environmental factors and greenhouse gas emissions is essential for evaluating and managing the climate impacts of aquaculture systems.

Despite the growing number of studies addressing greenhouse gas emissions from aquaculture, the influence of fish management practices on emission mitigation remains insufficiently understood. Evidence indicates that shrimp ponds emit more greenhouse gases than fishponds (Zhang et al., 2022), that aeration can reduce direct methane emissions (Fang et al., 2022), and that extensive and semi-intensive systems exhibit higher greenhouse gas emissions than intensive systems (Yuan et al., 2019). In integrated systems with benthivores species, most studies report no overall increase in greenhouse gas emissions (Belmudes et al., 2025; Flickinger et al., 2020a, 2019). In contrast, Oliveira Junior et al. (2019) reported that the inclusion of benthivorous species reduced methane emissions in ponds. The inclusion of benthic species has therefore emerged as a promising strategy for nutrient recovery and for reducing impacts related to eutrophication in aquaculture systems (Belmudes et al., 2025; Boyd et al., 2020; Flickinger et al., 2020a, 2019). However, the mechanisms linking species integration, sediment processes, and greenhouse gas dynamics remain poorly uncertain, especially in tropical environments. In this context, expanding pathway-specific analysis and temporally explicit assessments under different management strategies is essential to support the development of aquaculture practices that reconcile productivity with climate change mitigation.

In Brazil, tambaqui (*Colossoma macropomum*) is the most widely produced native fish species, with an annual production of approximately 970,000 t in 2024 (Peixe BR, 2025). Its cultivation is predominantly carried out in monoculture systems, in which formulated feed represents the main production input (Valenti et al., 2021). Tambaqui monoculture exhibits low nutrient recovery efficiency, retaining only about 18% of carbon, 15-21% of nitrogen, and 13-22% of phosphorus supplied to the system (Flickinger et al., 2020b, 2020a, 2019; Lima and Valenti, 2025). Consequently, unrecovered nutrients promote eutrophication and contribute to greenhouse gas emissions (Li et al., 2021). Integrated culture systems, by incorporating a greater proportion of nutrients into protein biomass (Belmudes et al., 2025; Thomas et al., 2020), may reduce eutrophication and influence greenhouse gas emissions. In addition, benthic species can modify sediment processes and have been associated with reduced methane emissions under certain management and environmental conditions (Oliveira Junior et al., 2019), which is one of the most impactful greenhouse gases emitted by aquaculture. Accordingly, in the present study, we included curimba (*Prochilodus lineatus*) in tambaqui production systems, integrating this benthic species into conventional tambaqui monoculture ponds. Curimba is a detritivorous, demersal fish that feeds primarily on organic detritus and represents the fifth most produced group of native species for human consumption in the country (Kalous et al., 2012; Valenti et al., 2021).

Therefore, this study aimed to evaluate the effects of integrating curimba (*Prochilodus lineatus*) into conventional tambaqui (*Colossoma macropomum*) monoculture systems on greenhouse gas emissions and biogeochemical processes in tropical aquaculture ponds. By simultaneously quantifying diffusive and ebullitive fluxes of $CO_2$, $CH_4$, $N_2O$, and $N_2$, and adopting a temporal approach based on monthly measurements throughout the production cycle, this study provides a comprehensive assessment of greenhouse gas dynamics under integrated and monoculture production systems and contributes to understanding the variability of gas flux measurements among replicates in aquaculture ponds.

## Material and Methods

### *Study site and pond preparation*

The experiment was carried out at the Aquaculture Experimental Center of Embrapa in Palmas, Tocantins, Brazil (10°08'01.33"S, 48°19'09.86"W), between July 2023 and February 2024. Six earthen ponds (600 m²; 1.3 m depth) were prepared by complete draining, followed by disinfection with quicklime (200 g $m^{-2}$). Subsequently, limestone (200 g $m^{-2}$) was applied, and fertilization was performed with urea (5 g $m^{-2}$), single superphosphate (3 g $m^{-2}$), and rice bran (10 g $m^{-2}$).

Water was supplied from a nearby reservoir. Throughout the seven-month production cycle, water replacement was minimal and limited to compensating for evaporation and seepage.

*Experimental design*

The study evaluated two aquaculture production systems in a randomized design with three replicates per treatment. The first treatment was a tambaqui monoculture (T), stocked at 0.4 fish $m^{-2}$, with an average initial body weight of 29 ± 4 g. The second was an integrated system (TC), in which tambaqui were stocked at the same density (0.4 fish $m^{-2}$) together with curimba at 0.3 fish $m^{-2}$, with average initial body weights of 29 ± 4 g and 15 ± 9 g for tambaqui and curimba, respectively (Figure 1). These stocking densities follow those typically applied during the commercial grow-out phase of tambaqui (Valenti et al., 2021). Curimba stocking density was adapted from previous experiments with this species in IMTA (e.g., Franchini et al., 2020), considering the relative proportions between the two species.

Tambaqui was the target species and was fed twice daily (08:00 and 16:00 h) with extruded commercial feed (Archer Daniels Midland Company – ADM, Descalvado, São Paulo, Brazil). Fish weighing 30–230 g, 230–410 g, and 410–700 g received a diet containing 32% crude protein at feeding rates of 4.5%, 3%, and 2% of body weight per day, respectively. For fish weighing 700–1,130 g and those above 1,130 g, a 28% crude protein diet was offered at feeding rates of 2% and 1.5% of body weight per day, respectively (Oliveira et al., 2013). Monthly, 30 tambaqui and 30 curimba were randomly sampled and weighed in each pond to monitor growth, after which they were returned to the water. Feed adjustments were based solely on tambaqui biomass. The apparent feed conversion ratio (FCR) was calculated as total feed supplied divided by weight gain.

Aeration (fountain-type, 1.5 HP) was applied whenever dissolved oxygen dropped below 3 mg/L, totaling 457 ± 392 hours over the production cycle, with no differences observed between treatments (*t*-test, $P > 0.05$). At the end of the experiment, ponds were drained, and all fish were harvested, counted, and weighed in batches of five individuals to determine total yield. Fish yield was calculated as final biomass per pond area and expressed in kg $year^{-1}$. Survival (%) was calculated at the end of the experimental period as the ratio between the number of fish harvested and the number of fish initially stocked, multiplied by 100.

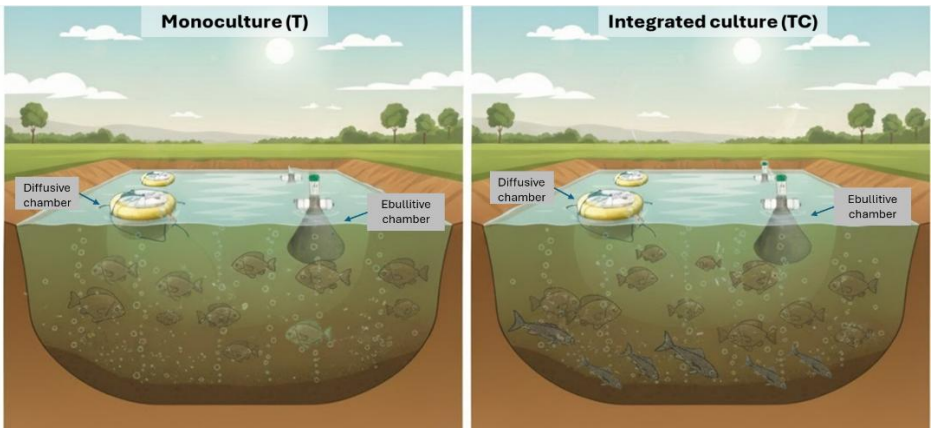


**Figure 1.** Schematic representation of the experimental design showing the two aquaculture systems evaluated: tambaqui (*Colossoma macropomum*) monoculture (T) and integrated system with curimba (*Prochilodus lineatu*s) (TC). Diffusive fluxes were measured using floating chambers, while ebullitive fluxes were captured with inverted funnels. Each pond was equipped with replicated chambers and funnels during all sampling events. Illustration generated using an artificial intelligence–based tool (Nano Banana, Gemini platform, Google).

*Water quality parameters*

Temperature, dissolved oxygen, conductivity, and pH were measured three times per week at 08:00 using a YSI Professional Plus multiparameter probe (Yellow Springs Instruments, Yellow Springs, USA). Monthly, each pond was sampled to determine chlorophyll-*a*, total suspended solids, dissolved solids, total nitrogen, total phosphorus, and turbidity. All analyses followed standard APHA procedures (APHA, 2017), except for turbidity, which was measured with a turbidimeter (Hach 2100Q, Loveland, CO, USA). Total organic carbon (dissolved and particulate), dissolved inorganic carbon (the sum of carbonates, bicarbonates, and carbon dioxide), and total nitrogen were determined using an elemental analyzer (Vario TOC Select, Elementar, Germany). In this process, samples underwent high-temperature catalytic oxidation, and the analytes were quantified using an infrared detector. For the determination of total inorganic carbon, samples were digested with 1% phosphoric acid.

*Sediment parameters*

Soil samples were collected monthly using a soil sampling auger. Three cores (10–15 cm depth) were taken from each pond and pooled into a composite sample. Samples were dried in a forced-air oven at 45 °C for 72 h, after which a subsample was taken for chemical analyses. Carbon and total nitrogen were determined using a CHNS elemental analyzer (Vario MACRO Cube, Elementar, Germany). Samples were subjected to high-temperature catalytic combustion, generating gaseous

products that were chromatographically separated according to their retention times. The separated gases were then directed to a thermal conductivity detector (TCD), which identified each compound based on differences in thermal conductivity. For phosphorus determination, samples were subjected to wet digestion with a nitric–perchloric acid mixture (3:1), and phosphorus concentrations in the resulting extract were quantified using the molybdate–vanadate spectrophotometric method (Silva, 2009).

*Diffusive and ebullitive flux measurement*

Diffusive and ebullitive emissions of $CO_2$, $CH_4$, $N_2O$, and $N_2$ were measured monthly using diffusion chambers and inverted funnels, respectively. Pond stocking was designated as month 0, representing baseline conditions. Due to operational constraints, no sampling was conducted in month 1. Therefore, temporal analyses include months 0, 2, 3, 4, 5, 6, and 7.

Diffusive fluxes were measured using two floating chambers per pond (each with a usable volume of 1 L and surface area of 0.049 m²) during the day (10:00–12:00) and at night (22:00–24:00) (Figure 1). Gas samples were collected immediately after sealing the chamber (defined as time 0), and subsequently at 1, 2, and 4 minutes after sealing. Samples were transferred to evacuated tubes and analyzed by gas chromatography (Shimadzu – GC-2014 Permanent Gas Analyzer). Carbon dioxide and methane were quantified using a thermal conductivity detector (TCD) and a flame ionization detector (FID) equipped with a methanizer, while nitrous oxide was quantified using an electron capture detector (ECD). Gas species were identified and quantified based on their retention times and detector responses. At each sampling event, air temperature and atmospheric pressure were obtained from the on-site meteorological station (INMET, 2025; station 83033). Gas fluxes were calculated in three steps: (1) estimating the number of moles inside the chamber, (2) converting moles to mass, and (3) quantifying fluxes from temporal changes in mass. The number of moles (n) was calculated from the ideal gas law:

$$n = \frac{P * V}{R * T}$$

where *P* is atmospheric pressure (atm), *V* is chamber volume (L), *R* is the gas constant (62.36 L atm $K^{-1}$ $mol^{-1}$), and *T* is air temperature (K). Mass (W, mg) was calculated as:

$$W = n * C * Wg * CF1$$

where *C* is gas concentration (ppm, ppb, and %), *Wg* is molar mass (mg), and C*F1* is the conversion factor used to express gas concentration as an absolute value

rather than a fraction ($10^{-2}$ for $N_2$, $10^{-6}$ for $CO_2$ and $CH_4$, and $10^{-9}$ for $N_2O$). Partial diffusive fluxes (*Fp*, mg m⁻² period⁻¹) were obtained as:

$$Fp = \frac{1}{N} * \left( \frac{(Wt1 - Wt0)}{(t1 - t0)} + \frac{(Wt2 - Wt1)}{(t2 - t1)} + \frac{(Wt4 - Wt2)}{(t4 - t2)} \right) * \frac{CF_2}{A}$$

Where $W_{t_i}$ is gas mass at times 0, 1, 2, and 4 min; *N* is the number of flux increments used; $CF_2$ converts mg m⁻² min⁻¹ to mg m⁻² day⁻¹; and A is chamber area (m²). Daily diffusive fluxes were obtained by summing daytime and nighttime partial fluxes.

Ebullitive emissions were measured monthly using two inverted fiberglass funnels per pond (each with a surface area of 0.0707 m²) positioned at approximately one-quarter of the pond length on opposite sides (Figure 1). Funnels were mounted on floating supports and anchored with a ~6-m rope, allowing limited movement within the pond. Gas bubbles accumulated over 48 h in a graduated collection bottle attached beneath each funnel. Collected gas was transferred to sampling tubes and analyzed by gas chromatography using the same GC system and procedures as for diffusive samples. Average daytime and nighttime temperature and pressure during the 48-h period were obtained from the on-site meteorological station (INMET, 2025; station 83033) for ebullitive flux calculations. Steps for estimating moles and mass (steps 1 and 2 above) were identical to those used for diffusive fluxes. Daily ebullitive flux ($F_{eb}$, mg m⁻² day⁻¹) was calculated as:

$$Feb = \frac{W}{(d * A)}$$

where *W* is the accumulated gas mass (mg), *d* is the duration of utilization (days), and *A* is the funnel area (m²).

*$CO_2$-equivalent*

The conversion of greenhouse gas emissions to $CO_2$-equivalent ($CO_2$-eq) was performed considering only the fluxes of $CH_4$ and $N_2O$, since biogenic $CO_2$ is not included in global warming metrics for aquatic production systems (Biermann and Geist, 2019; Hischier et al., 2010; Stichnothe and Hospido, 2008). Monthly fluxes of these gases were converted using the 100-year Global Warming Potentials (GWP) from the IPCC AR6, adopting values of 27.2 for methane ($CH_4$) and 273 for nitrous oxide ($N_2O$) (IPCC, 2021). Total $CO_2$-eq emissions were then obtained by summing the weighted contributions of both gases. These $CO_2$-eq values were then divided by the final fish biomass in each experimental unit, allowing the estimation of pond emission intensity expressed as $CO_2$-eq per kilogram of fish

produced. These estimates reflect only direct pond greenhouse gas emissions and should not be interpreted as full life-cycle emission intensities.

*Statistical analysis*

Before statistical analysis, all raw flux measurements were inspected for influential outliers using the *influence.measures()* function in R (R Core Team, 2025), which provides multiple diagnostic criteria for identifying influential observations. Based on these diagnostics, two $CO_2$, one $CH_4$, three $N_2O$, and four $N_2$ flux values were removed from the dataset.

For each sampling event, diffusive and ebullitive fluxes from the two replicate chambers or funnels within each pond were averaged, generating a single value per gas, per pathway, per pond, per month. The coefficient of variation (CV) for each chamber or funnels pair was calculated as 100 × (standard deviation/mean).

All datasets were screened for normality (Shapiro–Wilk test) and homogeneity of variances (Bartlett test). Box–Cox transformations were applied when appropriate. Fish mass, survival, yield, feed conversion ratio (FCR), and $CO_2$-equivalent emissions by fish biomass were compared between treatments using Student's *t*-test. To assess differences in diffusive, ebullitive, and total gas fluxes across treatments and months, we used repeated-measures ANOVA implemented as linear mixed-effects models. Treatment, month, and their interaction were included as fixed effects, and pond was included as a random intercept to account for repeated observations. When ANOVA indicated significant effects, treatment and temporal comparisons were performed using Tukey's post-hoc test. Statistical significance was considered at $P < 0.05$.

Pearson correlations were used to assess pairwise relationships between greenhouse gas fluxes and environmental variables. Correlation coefficients and associated *P*-values were obtained using the cor.test function in R. Analyses were performed separately for each gas and emission pathway, and only statistically significant correlations with $|r| \geq 0.35$ ($P < 0.05$) were reported. All remaining correlations, including non-significant results or those with $|r| < 0.35$, are provided as supplementary material (Tables S1, S2, and S3). All analyses and graphics were performed in R version 4.2.3 (R Core Team, 2025).

*Legal and Ethical Aspects*

The study complied with official Brazilian guidelines for the care and use of animals for scientific and educational purposes (Concea-CEUA protocol 76/2022), and with the National Management System for Genetic Heritage and Associated Traditional Knowledge (AB47B85).

## Results

*Fish performance*

Survival, growth performance, and feed efficiency of tambaqui were similar between monoculture (T) and the integrated system with curimba (TC), with no significant differences in tambaqui survival, yield, or FCR (Table 1). In the integrated ponds, curimba exhibited high survival (97 ± 6%) and contributed an additional 561 ± 179 kg $ha^{-1}$ to total production. Water quality conditions remained within acceptable ranges for both species throughout the culture period. Detailed water quality and sediment data are provided in Supplementary Table S4.

**Table 1.** Productive performance of tambaqui (*Colossoma macropomum)* monoculture (T) and integrated system with curimba (*Prochilodus lineatus*) (TC) in ponds. FCR – Feed conversion ratio.

| | T | TC | *P*-value* |
|---|---|---|---|
| *Tambaqui* | | | |
| Survival (%) | 58 ± 14 | 60 ± 14 | 0.8397 |
| Yield (kg/ha) | 3783 ± 383 | 4116 ± 550 | 0.4258 |
| FCR tambaqui | 1.73 ± 0.16 | 1.73 ± 0.25 | 0.9851 |
| *Curimba* | | | |
| Survival (%) | - | 97 ± 6 | - |
| Yield (kg/ha) | - | 561 ± 179 | - |

* *t*-test

*Variability between sample replicates*

The coefficients of variation of diffusive gas fluxes showed substantial dispersion across all measured gases (Figure 2). $CH_4$ exhibited the lowest variability (117 ± 128%). $CO_2$ displayed an intermediate level of variability (153 ± 147%), with a broader spread of CV values compared to $CH_4$. In contrast, $N_2$ (175 ± 164%) and $N_2O$ (179 ± 173%) showed the widest distributions, including numerous high CV values, reflecting the most heterogeneous emission patterns among the gases.

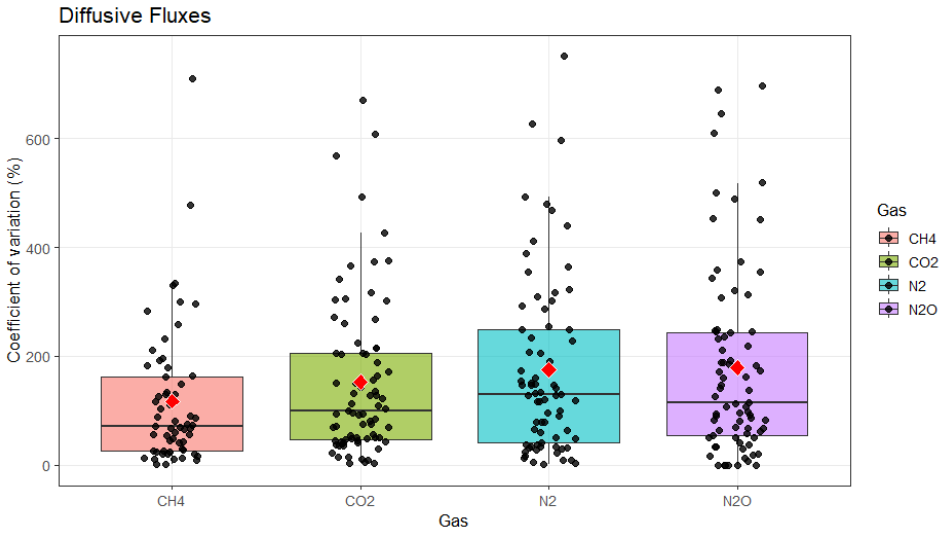


**Figure 2.** Distribution of coefficients of variation (CV) for diffusive fluxes of $CH_4$, $CO_2$, $N_2$ and $N_2O$. Each point represents a CV calculated from two replicate flux measurements collected in the same pond during each sampling event. Boxplots show the median and interquartile range for each gas, and red diamonds indicate mean CV values.

The ebullitive fluxes showed distinct variability patterns among gases (Figure 3). $CH_4$ exhibited the highest CV values (78 ± 45%), with a wide interquartile range and several high outliers, indicating pronounced variability in bubble-mediated emissions. $CO_2$ also showed relatively high variability (65 ± 41%), with a broad distribution but slightly lower dispersion than $CH_4$. In contrast, $N_2$ (32 ± 24%) and $N_2O$ (29 ± 22%) displayed much narrower distributions and lower CV values, reflecting more consistent ebullitive flux measurements. Overall, these results indicate that ebullition is substantially more variable for $CH_4$ and $CO_2$ than for $N_2$ and $N_2O$.

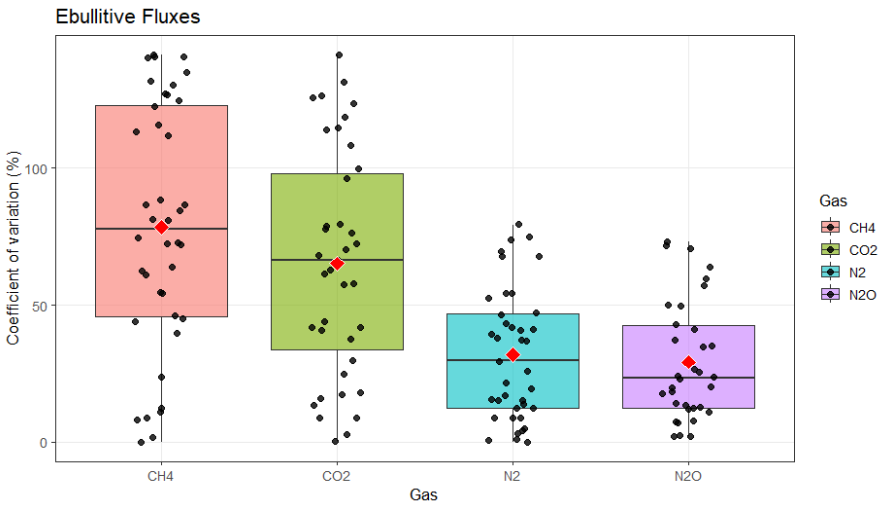


**Figure 3.** Distribution of coefficients of variation (CV) for ebullitive fluxes of $CH_4$, $CO_2$, $N_2$ and $N_2O$. Each point represents a CV calculated from two replicate flux measurements collected in the same pond during each sampling event. Boxplots show the median and interquartile range for each gas, and red diamonds indicate mean CV values.

*Spatiotemporal Patterns of $CO_2$ Emissions*

For $CO_2$ diffusive fluxes, TC ponds (317 ± 2207 mg $m^{-2}$ $day^{-1}$) generally exhibited net $CO_2$ emissions, whereas T ponds (−1041 ± 2409 mg $m^{-2}$ $day^{-1}$) showed net $CO_2$ uptake throughout most of the production cycle ($P$ = 0.0260) (Figure 4, Table 2). In contrast, ebullitive $CO_2$ fluxes were not influenced by treatment but varied significantly over time ($P$ = 0.0011), increasing toward the later months of the cycle (Figure 4). When diffusive and ebullitive components were combined, total $CO_2$ fluxes again showed a significant treatment effect ($P$ = 0.0258), with TC ponds exhibiting net $CO_2$ emissions (320 ± 2206 mg $m^{-2}$ $day^{-1}$) and T ponds showed net $CO_2$ uptake (−1039 ± 2409 mg $m^{-2}$ $day^{-1}$) throughout the study (Figure 4). No significant time effect was detected for total $CO_2$ fluxes ($P$ = 0.604). Full numerical datasets for diffusive, ebullitive, and total fluxes of all gases are presented in the supplementary material (Tables S5–S7).

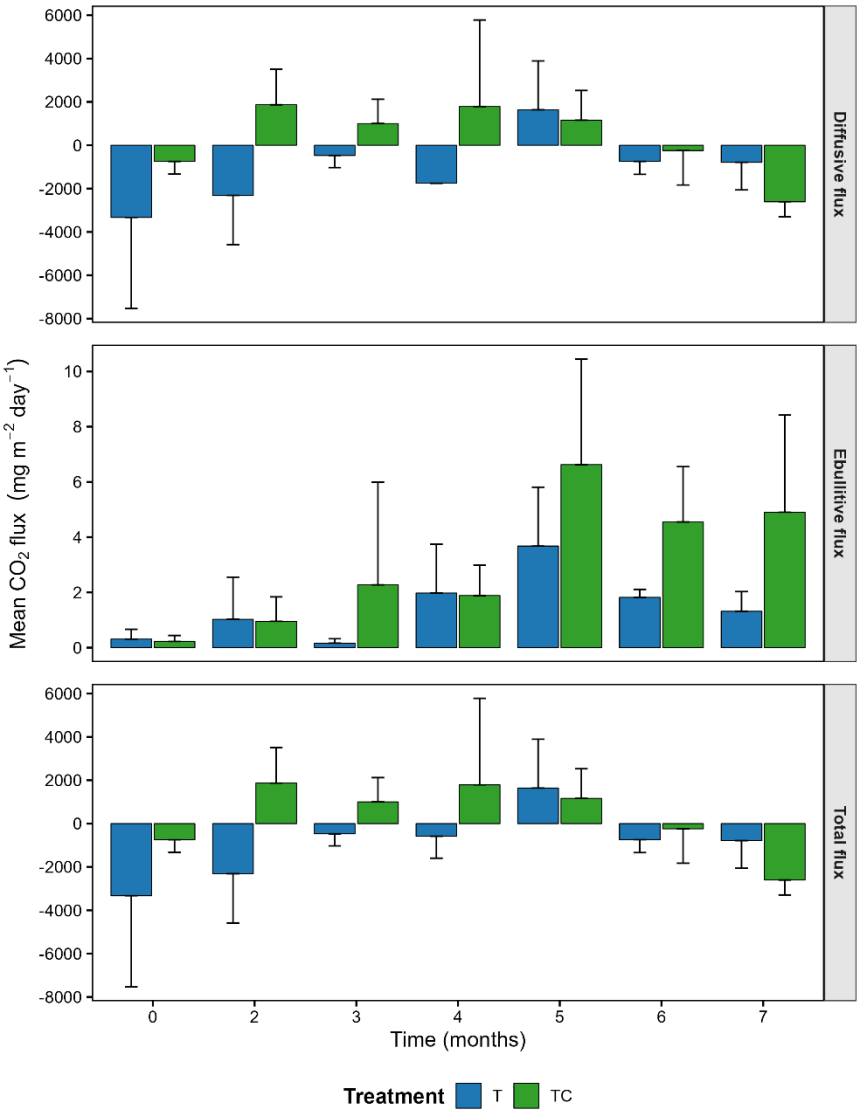


**Figure 4.** Mean diffusive, ebullitive, and total $CO_2$ fluxes over the production cycle of tambaqui (*Colossoma macropomum*) in monoculture (T) and integrated system with curimba (*Prochilodus lineatus*) (TC). Negative values indicate net $CO_2$ uptake, whereas positive values represent net emissions. Error bars represent standard deviations.

**Table 2.** Means (±SD) of gas fluxes (mg $m^{-2}$ $day^{-1}$) and $CO_2$-equivalent emissions in monoculture (T) and integrated system with curimba *Prochilodus lineatus* (TC), presented by diffusive, ebullitive, and total pathways. Different lowercase letters within the same row indicate significant differences between treatments according to Tukey's test ($p < 0.05$). Positive values indicate gas emissions from the pond to the atmosphere, while negative values indicate net gas uptake by the water column.

| Gas (mg $m^{-2}$ $day^{-1}$) | Pathway | T | TC | *P*-value | | |
|---|---|---|---|---|---|---|
| | | | | *Treat* | *Time* | *Treat*Time* |
| $CO_2$ | Diffusive | **−1041 ± 2409** [b] | **317 ± 2207** [a] | **0.0260** | 0.0608 | 0.1632 |
| | Ebullitive | 1.63 ± 1.53 | 3.21 ± 3.10 | 0.0652 | **0.0011** | 0.8958 |
| | Total | **−1040 ± 2409** [b] | **320 ± 2206** [a] | **0.0258** | 0.0604 | 0.1635 |
| $CH_4$ | Diffusive | 11.89 ± 33.11 | 19.16 ± 24.09 | 0.1159 | 0.0767 | 0.2375 |
| | Ebullitive | 38.53 ± 54.60 | 37.71 ± 48.77 | 0.9175 | **0.0390** | 0.3606 |
| | Total | 41.88 ± 64.44 | 55.92 ± 50.16 | 0.3970 | 0.0945 | 0.5186 |
| $N_2O$ | Diffusive | −0.03 ± 0.33 | 0.10 ± 0.39 | 0.2320 | 0.1540 | 0.4120 |
| | Ebullitive | 0.0005 ± 0.0009 | 0.0004 ± 0.0005 | 0.2650 | 0.0508 | 0.1866 |
| | Total | −0.03 ± 0.33 | 0.10 ± 0.38 | 0.2340 | 0.1540 | 0.4050 |
| $N_2$ | Diffusive | 7780 ± 28396 | 6146 ± 30664 | 0.8142 | 0.1721 | **0.0416** |
| | Ebullitive | 205 ± 180 | 254 ± 220 | 0.7842 | 0.5112 | 0.7704 |
| | Total | 7966 ± 28449 | 6384 ± 30704 | 0.8073 | 0.1694 | **0.0419** |
| $CO_2$ eq. | Total | 1132 ± 1760 | 1366 ± 1181 | 0.2146 | **0.0181** | 0.5785 |

### *Spatiotemporal Patterns of $CH_4$ Emissions*

Methane emissions showed distinct patterns between ebullitive and diffusive pathways. Diffusive $CH_4$ fluxes exhibited no significant effects of treatment or time and remained relatively low throughout the production cycle, typically below 40 mg $m^{-2}$ $day^{-1}$ (Figure 5, Table 2). In contrast, ebullitive $CH_4$ fluxes varied significantly over time ($P = 0.0390$), with peaks occurring mainly in mid-production months in both treatments (Figure 5). When ebullitive and diffusive components were combined, total $CH_4$ emissions also showed no significant treatment or temporal effects (Figure 5), indicating that temporal variability in ebullition did not translate into detectable differences in overall $CH_4$ release between monoculture (T) and integrated system with curimba (TC).

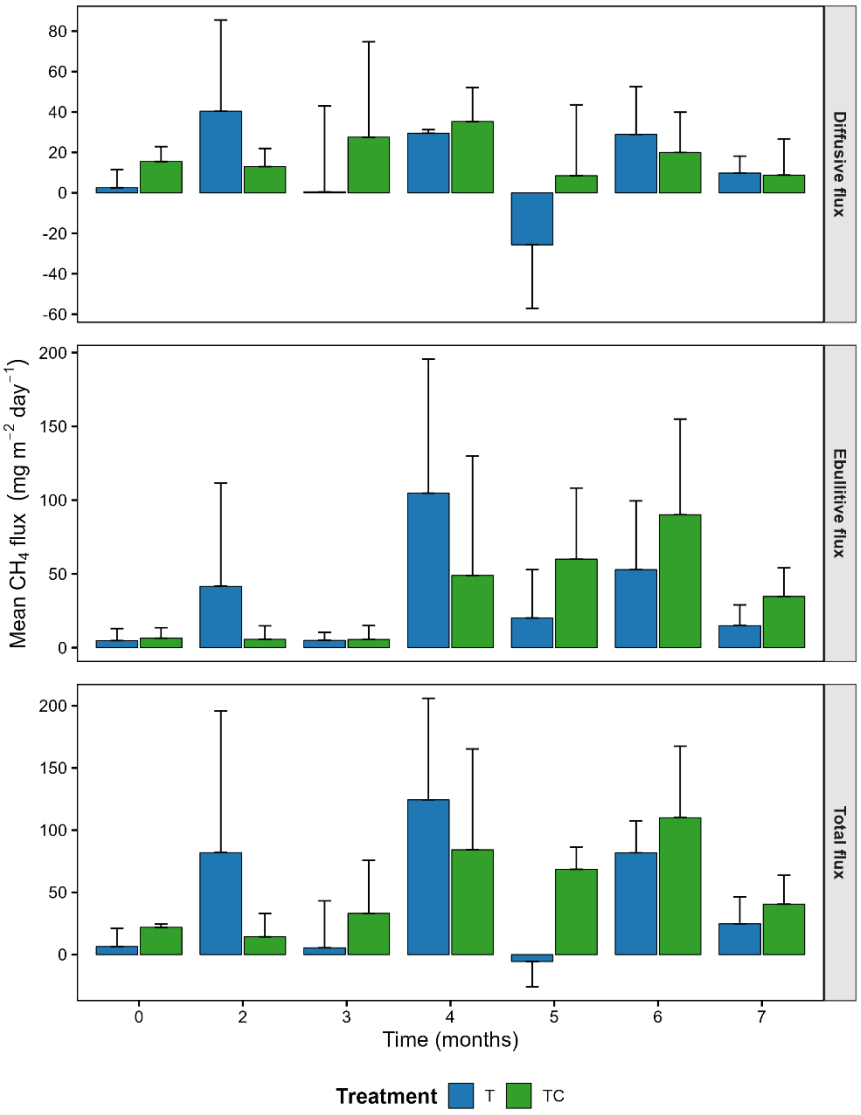


**Figure 5**. Mean diffusive, ebullitive, and total $CH_4$ fluxes over the production cycle of tambaqui (*Colossoma macropomum*) in monoculture (T) and integrated system with curimba (*Prochilodus lineatus*) (TC). Negative values indicate net $CH_4$ uptake, whereas positive values represent net emissions. Error bars represent standard deviations.

*Spatiotemporal Patterns of $N_2O$ Emissions*

Nitrous oxide fluxes showed no significant effects of treatment or time for either diffusive or ebullitive pathways ($P > 0.05$). Diffusive fluxes fluctuated around zero throughout the production cycle, with both monoculture (T) and integrated (TC) systems exhibiting similar temporal patterns (Figure 6, Table 2). Ebullitive $N_2O$ emissions were consistently low, and although values tended to increase slightly at certain months in both treatments, the time effect approached but did not reach

statistical significance ($P$ = 0.0508) (Figure 6). When diffusive and ebullitive components were combined, total $N_2O$ fluxes likewise showed no significant effects of treatment or time ($P$ > 0.05) (Figure 6), indicating that $N_2O$ emissions remained low, stable, and similar between monoculture and integrated system.

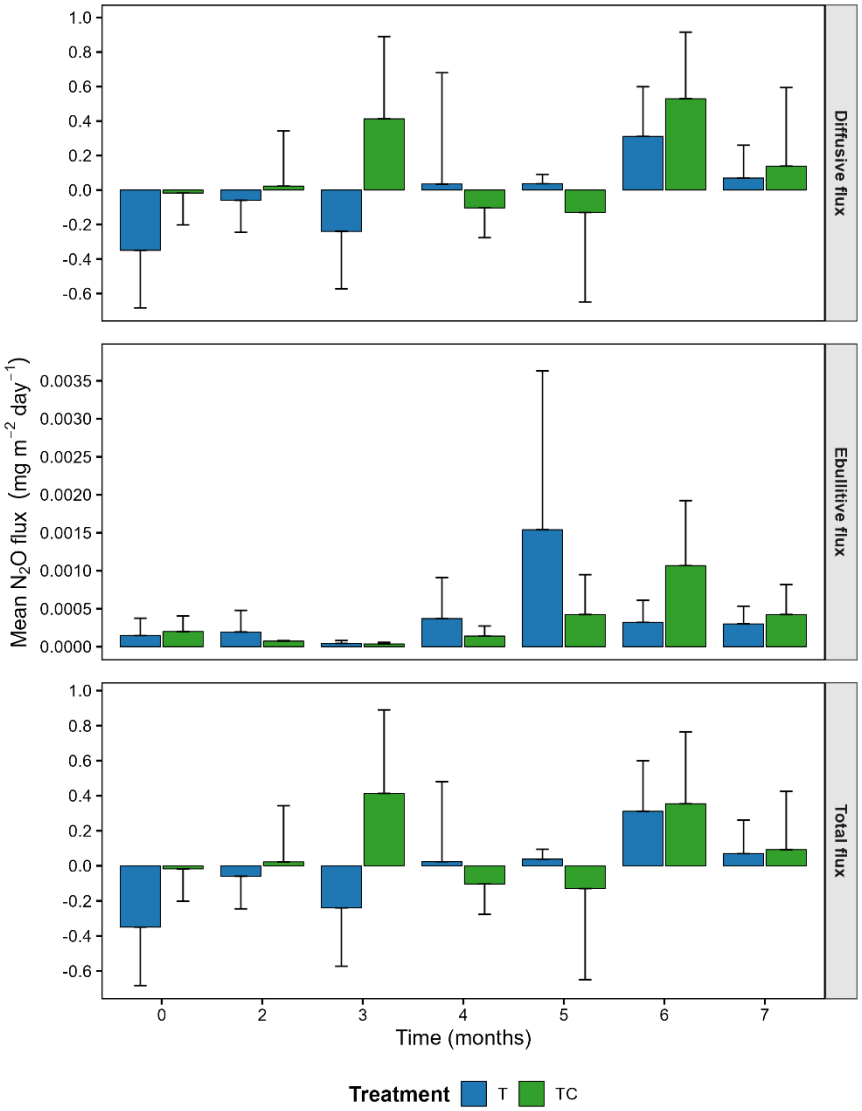


**Figure 6**. Mean diffusive, ebullitive, and total $N_2O$ fluxes over the production cycle of tambaqui (*Colossoma macropomum*) in monoculture (T) and integrated system with curimba (*Prochilodus lineatus*) (TC). Negative values indicate net $N_2O$ uptake, whereas positive values represent net emissions. Error bars represent standard deviations.

*Spatiotemporal Patterns of $N_2$ Emissions*

Nitrogen fluxes showed distinct patterns between diffusive, ebullitive, and total pathways. For diffusive $N_2$ fluxes, there was a significant interaction between treatment and time ($P$ = 0.0416). Differences between monoculture (T) and integrated culture (TC) were detected at months 3 and 5 (Figure 7, Table 2). At month 3, TC exhibited pronounced negative fluxes (net $N_2$ uptake), whereas at month 5 the negative fluxes were observed in T. For ebullitive $N_2$ fluxes, no significant treatment or time effects were observed ($P$ > 0.05) (Figure 7), indicating similar temporal patterns of bubble-mediated $N_2$ release between the two production systems. For total $N_2$ fluxes (diffusive + ebullitive), there was again a significant interaction between treatment and time ($P$ = 0.0419) (Figure 7). As in the diffusive component, differences between T and TC occurred in months 3 and 5, driven primarily by the contrasting diffusive fluxes during these periods.

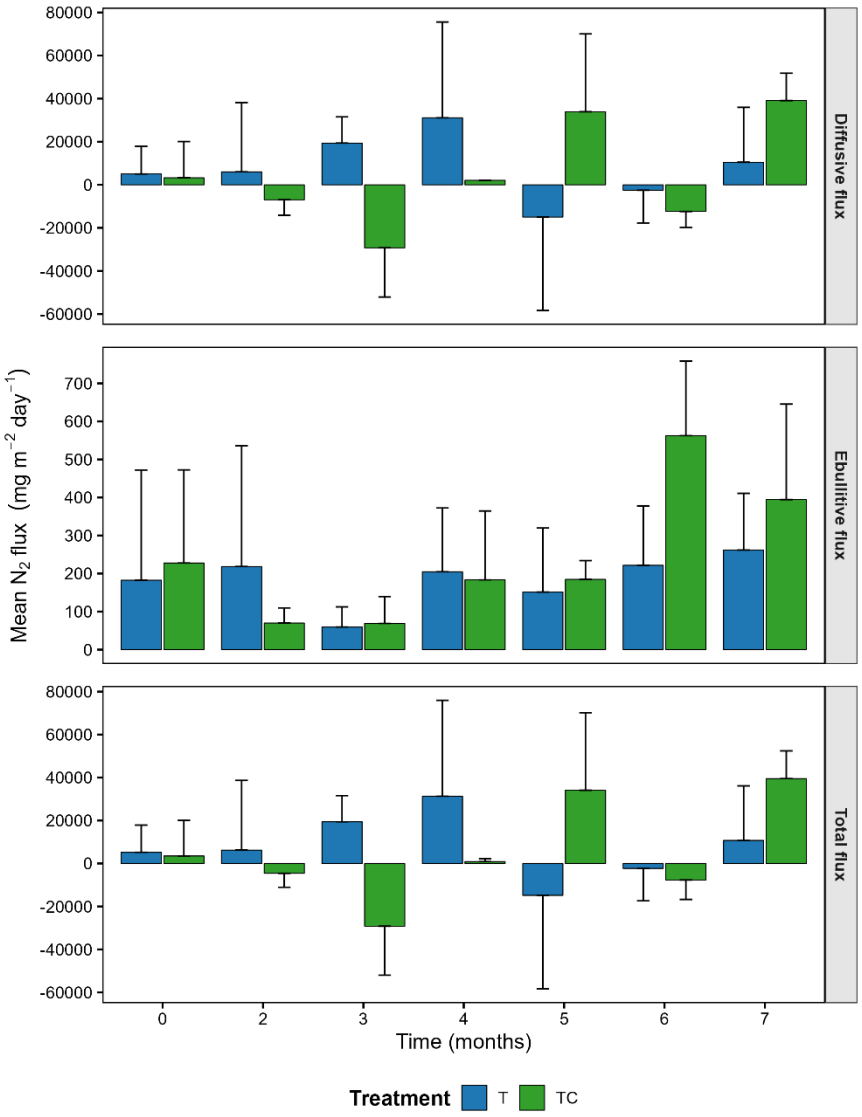


**Figure 7.** Mean diffusive, ebullitive, and total $N_2$ fluxes over the production cycle of tambaqui (*Colossoma macropomum*) in monoculture (T) and integrated culture with curimba (*Prochilodus lineatus*) (TC). Negative values indicate net $N_2$ uptake, whereas positive values represent net emissions. Error bars represent standard deviations.

*Spatiotemporal Patterns of Total $CO_2$ eq. Emissions*

Total pond $CO_2$-equivalent emissions, calculated from $CH_4$ and $N_2O$ fluxes only (biogenic $CO_2$ excluded), varied significantly over the production cycle ($P$ = 0.0181) (Figure 8, Table 2). Emission rates were relatively low at the beginning of the cycle and increased toward mid-cycle, with the highest values observed in months 4 and 6. This temporal increase was primarily driven by higher $CH_4$ emissions from the ebullitive pathway, which rose toward the mid- and late stages of the cycle. In

contrast, diffusive $CH_4$ and both diffusive and ebullitive $N_2O$ remained relatively stable over time. No treatment effects were detected ($P > 0.05$), and therefore results are presented pooled across treatments in Figure 8.

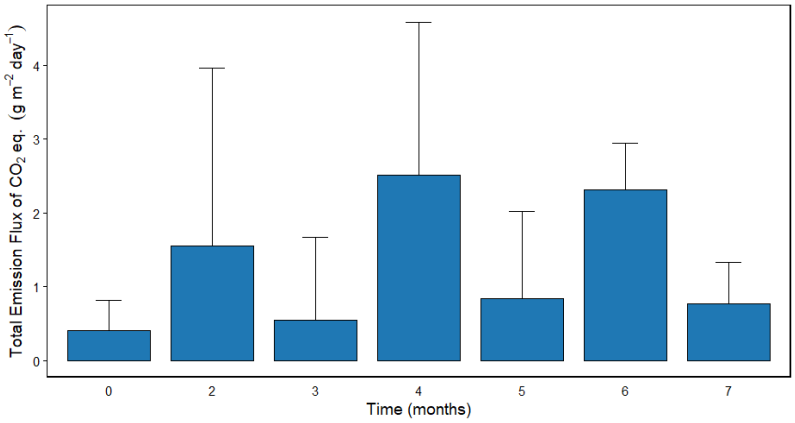


**Figure 8.** Total emission flux of $CO_2$ equivalents (g $m^{-2}$ $day^{-1}$) across the production cycle, calculated from $CH_4$ and $N_2O$ fluxes only. A significant effect of time was detected ($P = 0.0214$), with higher emissions in months 4 and 6. No treatment effect was observed ($P > 0.05$), so data were pooled across treatments.

*Greenhouse Gas Emissions ($CO_2$-eq) per Unit of Fish Production*

Greenhouse gas emissions per kilogram of fish produced did not differ significantly between treatments ($P = 0.5900$; Figure 9). The average emission intensity was slightly lower in the monoculture ponds (T) (0.61 ± 0.24 kg of $CO_2$ eq. kg $fish^{-1}$) than in the integrated ponds (TC) (0.72 ± 0.17 kg of $CO_2$ eq. kg $fish^{-1}$). These results indicate that, under the conditions of this experiment, integrating curimba into tambaqui culture did not significantly alter the emission intensity per unit of fish produced.

All numerical values of $CO_2$, $CH_4$, $N_2O$, and $N_2$ emissions, including diffusive, ebullitive, total fluxes, and $CO_2$-equivalent emissions, are provided in the supplementary material (Tables S5, S6, and S7).

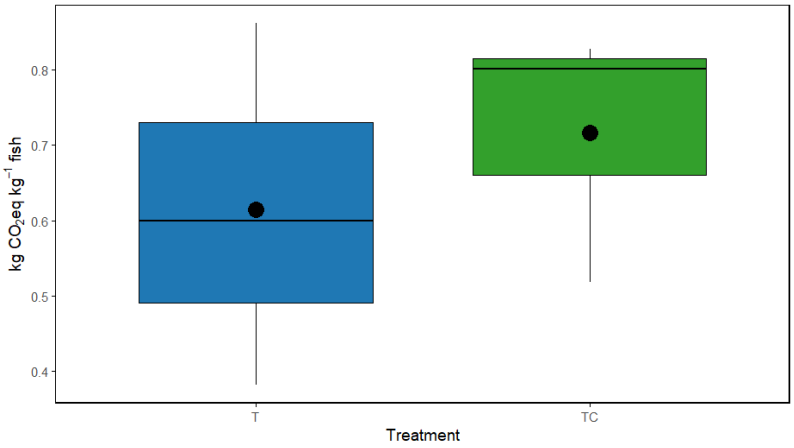


**Figure 9.** Emission intensity expressed as kg $CO_2$-eq per kg of fish produced in ponds managed under tambaqui (*Colossoma macropomum*) monoculture (T) and integrated culture with curimba (*Prochilodus lineatus*) (TC). Values represent only direct greenhouse gas emissions at the pond–atmosphere interface and do not include other sources typically considered in life cycle assessments. The black dot represents the mean and the horizontal line the median.

*Relative contribution of diffusive and ebullitive fluxes*

The relative contribution of diffusive and ebullitive fluxes varied substantially among gases (Figure 10). Methane ($CH_4$) was the only gas for which ebullitive emissions dominated, with ebullition accounting for 57.6 ± 24.8% of total $CH_4$ fluxes, whereas diffusion contributed 42.4 ± 24.8%. In contrast, emissions of $CO_2$, $N_2$ and $N_2O$ were almost entirely diffusive. Diffusive $CO_2$ fluxes represented 99.7 ± 0.47% of total emissions, with ebullition contributing less than 0.3%. Similarly, $N_2O$ diffusive fluxes comprised 99.4 ± 1.1%, while ebullitive contributions were negligible (0.55 ± 1.09%). $N_2$ was emitted predominantly through diffusion (97.5 ± 2.9%), and ebullition accounted for only 2.5 ± 2.9%.

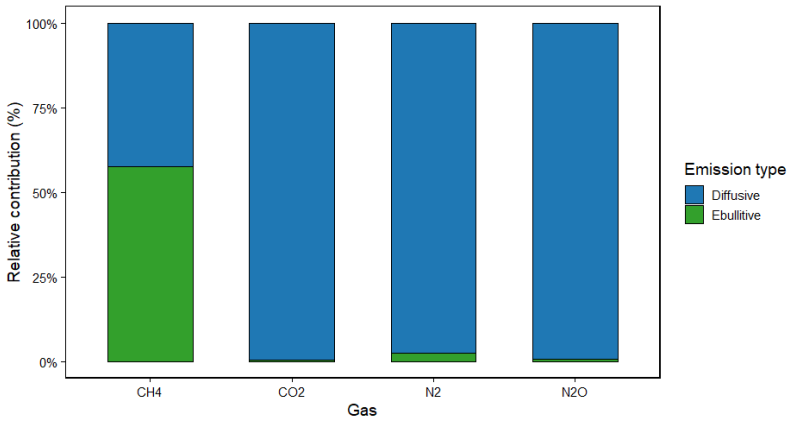


**Figure 10.** Relative contribution (%) of diffusive and ebullitive fluxes to total emissions of $CH_4$, $CO_2$, $N_2$, and $N_2O$ in the experimental ponds. Diffusive fluxes dominated the emissions of all gases, while ebullition represented a substantial share only for $CH_4$.

The relative contribution of diffusive and ebullitive pathways to total $CH_4$ emissions varied markedly over the production cycle in both culture systems (Figure 11). Although the dominance of each pathway shifted temporally, the overall proportions were similar between monoculture and integrated ponds, indicating that curimba integration did not substantially modify the dominant $CH_4$ emission pathway.

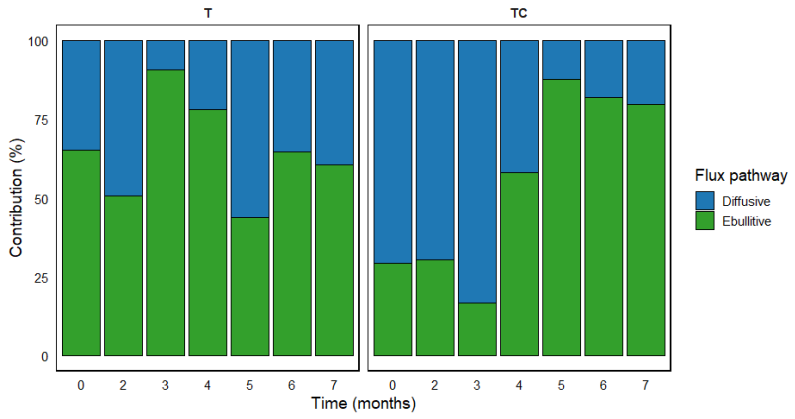


**Figure 11**. Relative contribution of diffusive and ebullitive $CH_4$ fluxes throughout the production cycle in ponds used for tambaqui (*Colossoma macropomum*) monoculture (T) and integrated culture with curimba (*Prochilodus lineatus*) (TC). Bars represent the proportional (%) contribution of each emission pathway to total $CH_4$ flux at each sampling month.

*Environmental drivers of gas fluxes*

Significant correlations ($|r| \geq 0.35$; $P < 0.05$) revealed contrasting controls on greenhouse gas fluxes depending on the emission pathway (Table 3). Diffusive $CO_2$ fluxes were primarily associated with water alkalinity, hardness, and dissolved inorganic carbon, whereas ebullitive $CH_4$ fluxes were more strongly related to particulate matter and nutrient availability. $N_2O$ fluxes were negatively correlated with sediment nitrogen, whereas $N_2$ fluxes showed significant associations with sediment and water variables. Soil nutrient pools were negatively correlated with $CO_2$-equivalent emissions. All remaining correlations are provided as supplementary material (Tables S1, S2, and S3).

**Table 3**. Significant Pearson correlations (|r| ≥ 0.35, $p < 0.05$) between greenhouse gas fluxes and environmental variables.

| Gas flux (pathway) | Environmental variable | r | *P*-value |
|---|---|---|---|
| $CH_4$ (total) | Total phosphorus (water) | 0.47 | 0.011 |
| $CH_4$ (ebullitive) | Total phosphorus (water) | 0.56 | <0.001 |
| | Total suspended solids | 0.50 | 0.001 |
| | Turbidity | 0.35 | 0.027 |
| | Chlorophyll-a | 0.35 | 0.028 |
| $CO_2$ (total) | Water alkalinity | 0.60 | <0.001 |
| | Water hardness | 0.58 | <0.001 |
| | Dissolved inorganic carbon (water) | 0.51 | 0.001 |
| | Total carbon (water) | 0.47 | 0.003 |
| | Total dissolved solids | 0.35 | 0.033 |
| $CO_2$ (diffusive) | Water alkalinity | 0.55 | <0.001 |
| | Water hardness | 0.51 | <0.001 |
| | Dissolved inorganic carbon (water) | 0.45 | 0.004 |
| | Total carbon (water) | 0.41 | 0.009 |
| $CO_2$ (ebullitive) | Total suspended solids | 0.41 | 0.010 |
| | Turbidity | 0.36 | 0.024 |
| $CO_2$eq | Soil phosphorus | −0.60 | 0.007 |
| | Soil nitrogen | −0.54 | 0.018 |
| | Soil carbon | −0.46 | 0.048 |
| $N_2O$ (total) | Soil nitrogen | −0.43 | 0.042 |
| $N_2$ (total) | Soil phosphorus | 0.43 | 0.035 |
| $N_2$ (diffusive) | Soil nitrogen | −0.43 | 0.031 |
| $N_2$ (ebullitive) | Total dissolved solids | −0.38 | 0.017 |

## Discussion

*Methodological limitations and choice of flux calculation approach*

Different methods for calculating gas fluxes from field measurements using static chambers or gas collectors have been widely reported in the literature, with ongoing discussions regarding the advantages and limitations of each approach (Malyan et al., 2022; Minamikawa et al., 2015; Venterea et al., 2020). Although regression-based methods are the most applied, they are not always appropriate, particularly under conditions of low data linearity (Minamikawa et al., 2015). This was the case in the present study, where the relationship between gas concentration and time did not meet the linearity assumptions required for linear regression models, justifying the use of a concentration-difference approach. Although this approach may result in errors approximately 11% higher than those associated with regression-based methods, it is considered more suitable under low-linearity conditions and provides transparent and reproducible flux estimates under field constraints (Minamikawa et al., 2015; Venterea et al., 2020). Other studies, such as Singh et al. (2005) and Vroom et al. (2023), also estimated gas fluxes using concentration changes over time. In the present study, diffusive fluxes were calculated as the average of multiple consecutive concentration increments, improving the robustness of flux estimates.

*Variability between sample replicates*

The high coefficients of variation observed among sample replicates in the present study reflect the heterogeneity of greenhouse gas emissions in aquatic environments. Although the assessment of CVs among replicates collected at the same time and within the same experimental unit has been relatively underexplored in aquatic GHG studies, substantial variability is well documented in terrestrial systems. For instance, differences among soil replicates have been reported to vary by up to 54-fold, with CVs ranging from 11% to 2000% for $N_2O$ fluxes (Chadwick et al., 2014). Similarly, Borjesson et al. (2000) reported CVs of 343% and 386% for methane emissions measured in landfill environments. These findings highlight that high CV values are a common feature of gas flux measurements, driven by microscale heterogeneity, episodic emission events, and dynamic biogeochemical processes. Therefore, the variability observed in the present study is consistent with previous reports and does not indicate methodological artifacts but rather reflects the natural variability of gas exchange processes in aquatic production systems.

### *$CO_2$ Emissions*

The magnitude of $CO_2$ fluxes in both systems (320 ± 2206 mg $m^{-2}$ $day^{-1}$ in TC and −1039 ± 2409 mg $m^{-2}$ $day^{-1}$ in T) falls within the wide range reported for aquaculture ponds and other eutrophic environments (−3828 to 5544 mg $m^{-2}$ $day^{-1}$;Malyan et al., 2022; Waldemer and Koschorreck, 2023), indicating that the fluxes observed in this study are representative of typical $CO_2$ dynamics in managed aquatic ecosystems while also reflecting the high natural variability of $CO_2$ exchange in freshwater systems. In addition, the alternation between $CO_2$ emission and uptake observed in this study is consistent with patterns commonly reported for freshwater systems (Barreto et al., 2024; Yang et al., 2018; Zhang et al., 2022).

The integrated system behaved, on average, as a net $CO_2$ source, whereas tambaqui monoculture behaved predominantly as a $CO_2$ sink. This contrast indicates that $CO_2$ dynamics were controlled by differences in biogeochemical conditions between systems. Flickinger et al. (2020a) also observed increased $CO_2$ emissions, mainly via ebullition, in integrated systems relative to shrimp monoculture, whereas no differences were detected in comparison with fish monoculture. Although systems with similar nutrient inputs would be expected to exhibit comparable gas emissions (Kosten et al., 2020), the higher $CO_2$ emissions observed in TC ponds were associated with greater nutrient availability (N in water and N and P in sediments), higher chlorophyll concentrations, and increased suspended and dissolved solids. Similar relationships were reported by Chen et al. (2016) for polyculture ponds and are consistent with the general pattern described by Li et al. (2021), who identified eutrophic status, indicated by chlorophyll-*a* and nutrient concentrations, as a major driver of greenhouse gas emissions in freshwater ecosystems.

The higher nutrient availability in the integrated system was likely driven by curimba bioturbation, as reflected by increased turbidity and particulate matter, which re-mobilized sedimented nutrients into the water column. Although part of the nutrients incorporated into curimba biomass, the integrated system exhibited higher nutrient concentrations in water and sediments, indicating that benthivores fish enhanced nutrient recycling rather than promoting net nutrient removal. In contrast, in the monoculture system, nutrients were likely preferentially lost through water infiltration, which is more pronounced in ponds without benthivores fish (Belmudes et al., 2025; Lima et al., 2026). Nutrient enrichment in integrated ponds likely stimulated phytoplankton blooms, increasing organic matter deposition to sediments and fueling microbial respiration. As a result, the integrated system exhibited higher overall respiration rates driven by the combined activity of fish,

plankton, and sediment microbial communities, leading to reduced dissolved oxygen and a predominance of $CO_2$ emissions. In contrast, the monoculture system maintained lower nutrient and organic matter availability, favoring $CO_2$ undersaturation and net atmospheric uptake. Similar mechanisms have been described by Kosten et al. (2020), who highlighted that uneaten feed, fish feces, and primary production contribute substantial labile organic matter to pond sediments, enhancing heterotrophic respiration and $CO_2$ release under nutrient-enriched conditions (Chen et al., 2016; Li et al., 2021). The role of benthivores fish in regulating $CO_2$ dynamics is further supported by Oliveira Junior et al. (2019), who showed that bioturbation enhances organic matter decomposition under oxic conditions and $CO_2$ release.

Carbon dioxide dynamics were more strongly associated with physical and chemical parameters than with purely biological factors. Alkalinity and hardness were the strongest predictors of $CO_2$ fluxes, indicating that the carbonate buffering system played a central role in regulating $CO_2$ saturation and evasion. This pattern is consistent with Feijoó et al. (2022), who linked higher alkalinity to increased $CO_2$ in freshwater systems. The strong association with electrical conductivity further supports this chemical control. In addition, the positive relationship with water temperature reflects both reduced gas solubility and enhanced microbial respiration at higher temperatures. Together, these results indicate that biological processes primarily regulate $CO_2$ production within ponds, whereas physical and chemical conditions determine the magnitude of $CO_2$ emission to the atmosphere. Accordingly, $CO_2$ fluxes in aquaculture ponds emerge from the combined effects of nutrient loading, sediment processes, plankton dynamics, and carbonate chemistry, rather than biological productivity alone.

No significant temporal variation in $CO_2$ fluxes was observed during the experimental period, suggesting that the main drivers of $CO_2$ exchange remained relatively stable over time. The absence of significant temporal variation in $CO_2$ fluxes was accompanied by similarly stable nutrient concentrations in the system (N in water and N and P in sediments; Supplementary Figures S3, S4), indicating that nutrient driven biogeochemical processes remained relatively constant. This temporal stability occurred despite the progressive increase in feed input throughout the culture cycle, suggesting that enhanced nutrient recycling, assimilation by biota, and sediment retention buffered the system against short-term nutrient accumulation (Boyd, 1995).

Diffusive flux was the dominant pathway, accounting for more than 99% of total $CO_2$ exchange, while ebullitive fluxes contributed less than 1% of the total, a pattern also reported for fishponds by Waldemer and Koschorreck (2023) and

shrimp ponds by Barreto et al. (2024). Due to its high solubility in water, $CO_2$ tends to accumulate in the dissolved phase, and its exchange with the atmosphere is primarily governed by diffusive gradients across the water–air interface (Boehrer et al., 2021). This characteristic explains the dominance of diffusive fluxes observed in this study. Accordingly, the alternation between $CO_2$ uptake and emission observed in this study likely reflects shifts between undersaturated and supersaturated $CO_2$ conditions in the water column. Waldemer and Koschorreck (2023) similarly reported $CO_2$ uptake under undersaturated conditions and emissions under supersaturated conditions.

Despite its quantitative dominance in carbon fluxes, $CO_2$ is often less emphasized in greenhouse gas assessments of aquaculture systems because it is commonly considered biogenic and part of a short-term carbon cycle (Matuštík and Kočí, 2022; Stichnothe and Hospido, 2008). Consequently, $CO_2$ emissions are frequently excluded from environmental impact evaluations, which tend to prioritize $CH_4$ and $N_2O$ due to their higher global warming potentials (Biermann and Geist, 2019; Lima et al., 2026). Nevertheless, quantifying $CO_2$ fluxes remains essential to understand carbon processing, metabolic balance, and biogeochemical functioning in managed aquatic ecosystems such as aquaculture ponds.

*$CH_4$ Emissions*

Methane emissions differed between pathways. Diffusive $CH_4$ fluxes remained low, stable, and unaffected by treatment or time, whereas ebullitive fluxes varied significantly over the production cycle, with peaks in mid- to late-production months. Nevertheless, total $CH_4$ emissions showed no significant temporal or treatment effects, indicating that variability in ebullition did not translate into differences in overall methane release between systems. The magnitude of total $CH_4$ emissions observed in this study (41.499–55.9 mg $m^{-2}$ $day^{-1}$) agrees with values reported for freshwater aquaculture ponds, which typically range from 22.5 to 595 mg $m^{-2}$ $day^{-1}$ (Waldemer and Koschorreck, 2023), confirming that our results are representative of methane dynamics in managed pond systems. Oliveira Junior et al. (2019) reported reduced $CH_4$ and increased $CO_2$ emissions in ponds stocked with benthivores fish, however their comparison involved systems with and without fish. In contrast, because both treatments in our study included fish, the addition of a benthivores species did not significantly alter methane emissions, as also observed by Flickinger et al. (2020a). Instead, its influence was more clearly expressed in $CO_2$ dynamics, suggesting that benthic disturbance primarily shifts carbon processing pathways rather than overall carbon mineralization rates.

Although sediment nitrogen has been associated with increased $CH_4$ emissions in eutrophic systems (Vroom et al., 2023), this effect appears to depend on concurrent organic carbon accumulation (Yuan et al., 2021). In our study, sediment carbon did not differ between treatments, despite higher nutrient levels in the integrated system, explaining the similar $CH_4$ emissions and reinforcing the dominant role of organic carbon in regulating methane production (Yuan et al., 2021). Furthermore, although dissolved oxygen concentrations were slightly lower in the integrated system, this reduction was not sufficient to enhance methanogenesis. Bioturbation and sediment resuspension promoted by fish activity may have increased sediment oxygenation and stimulated $CH_4$ oxidation, thereby neutralizing potential increases in methane production (Oliveira Junior et al., 2019; K. Zhang et al., 2024).

The occurrence of net diffusive $CH_4$ uptake in one month represents a less frequently reported behavior in ponds, yet one that can be explained by shifts in the balance between methane production and oxidation. A similar pattern was reported by (Barreto et al., 2024) in shrimp ponds and by Yuan et al. (2021) in crab water ponds. While ponds are generally methane sources, a temporary dominance of oxidation over production can reverse diffusive fluxes. According to Sikar et al. (2019), when methane availability declines and oxidation increases, diffusive fluxes may reverse.

The dominance of ebullition in $CH_4$ emissions observed in this study is consistent with previous reports indicating that this pathway typically contributes 50–90% of total methane fluxes in aquaculture ponds (Davidson et al., 2018; Malyan et al., 2022; Waldemer and Koschorreck, 2023; Yang et al., 2018). In this study, ebullition accounted for 57.6 ± 24.8% of total $CH_4$ emissions, reflecting methane's low solubility in water and its accumulation in sediments prior to bubble release (Kosten et al., 2020). The relative contribution of ebullitive fluxes increased over the production cycle in both systems, particularly in the integrated ponds, coinciding with rising water temperature and declining dissolved oxygen (Supplementary Figure S1, S2). These conditions favor methanogenesis while limiting methane oxidation, thereby enhancing ebullitive release (Barreto et al., 2024; Davidson et al., 2018; Fang et al., 2022; Li et al., 2021). Similar responses to nutrient enrichment and warming have been reported by Davidson et al. (2018) who showed that ebullition becomes the dominant methane pathway under eutrophic and warmer conditions. Together, these results highlight ebullition as the primary and most dynamic pathway regulating $CH_4$ emissions in freshwater aquaculture systems.

Correlational analyses further supported this pathway-specific behavior. Diffusive $CH_4$ fluxes showed no significant relationships with environmental variables, consistent with Davidson et al. (2018), who also reported no clear link between eutrophication or increased nutrient availability and enhanced diffusive methane exchange. In contrast, ebullitive fluxes were positively correlated with chlorophyll-*a*, turbidity, suspended solids, and phosphorus in the water. These associations suggest that increased primary production and particulate organic matter deposition enhance the accumulation of labile organic substrates in sediments, favoring methanogenesis and subsequent bubble formation. A similar link between sediment nutrient accumulation and ebullitive $CH_4$ emissions was reported by Li et al. (2021) and Vroom et al. (2023), who demonstrated that eutrophication and sediment nitrogen enrichment stimulate methane production by increasing organic carbon availability for anaerobic microbial processes.

Overall, these results indicate that methane emissions in aquaculture ponds are largely controlled by processes regulating organic carbon supply to sediments, which preferentially enhance ebullitive $CH_4$ release. In contrast, diffusive methane fluxes remain comparatively stable and weakly responsive to short-term environmental changes. These results indicate that methane dynamics in aquaculture systems are more strongly controlled by carbon availability and redox conditions than by nutrient enrichment alone. Moreover, accurate assessment of methane emissions requires integrating diffusive and ebullitive pathways, as they respond differently to environmental drivers. The pronounced temporal variability of ebullition further highlights that infrequent measurements may underestimate total $CH_4$ emissions, underscoring the need for pathway-resolved, high-frequency monitoring throughout the production cycle.

*$N_2O$ Emissions*

Nitrous oxide fluxes remained low, stable, and comparable between tambaqui monoculture and the integrated system throughout the grow-out period, with mean values ranging from a slight uptake (−0.03 mg $N_2O$ $m^{-2}$ $day^{-1}$) to weak emissions (0.10 mg $N_2O$ $m^{-2}$ $day^{-1}$). Both uptake and emission phases were observed, driven mainly by diffusive fluxes, while ebullitive contributions were negligible, confirming that $N_2O$ exchange in aquaculture ponds is dominated by diffusion, as also reported by Waldemer and Koschorreck (2023). These magnitudes fall within the range reported in the review of aquaculture ponds, urban ponds, and other inland water bodies by Waldemer and Koschorreck (2023) (−0.53 to 0.67 mg $N_2O$ $m^{-2}$ $day^{-1}$), reinforcing that fishponds typically act as weak and highly dynamic components to $N_2O$ exchange, shifting between source and sink behavior over time.

Although higher sediment nitrogen has been associated with increased $N_2O$ emissions in eutrophic systems (Li et al., 2021; Singh et al., 2005; L. Zhang et al., 2024), such effects are strongly mediated by organic matter availability and redox conditions (Yuan et al., 2021). In the present study, despite higher nitrogen concentrations in the integrated system, sediment carbon levels did not differ between treatments (Supplementary Table S4), which likely limited denitrification intensity and explains the absence of differences in $N_2O$ emissions. Under these substrate-limited and strongly reducing conditions, $N_2O$ production tends to be reduced, while complete denitrification favors the reduction of $N_2O$ to $N_2$, promoting net $N_2O$ consumption (Barreto et al., 2024; Webb et al., 2019; Yuan et al., 2021). This interpretation is supported by the weak and negative correlation between total $N_2O$ fluxes and sediment nitrogen. Bioturbation by benthic fish may further contribute to this pattern by enhancing sediment oxygen penetration and stimulating $N_2O$ reduction to $N_2$, thereby neutralizing potential increases in $N_2O$ production. Similar mechanisms have been described by Zhang et al. (2024) and Oliveira Junior et al. (2019), who showed that sediment disturbance can suppress $N_2O$ accumulation while enhancing complete denitrification. Accordingly, the $N_2O$ uptake observed in both systems agrees with findings indicating that small artificial water bodies frequently behave as atmospheric sinks for $N_2O$, even under eutrophic conditions (Webb et al., 2019). Comparable magnitudes and alternating uptake–emission patterns have also been reported in fishponds using both diffusive-based approaches (Xiao et al., 2023) and measurements distinguishing diffusive and ebullitive pathways (Waldemer and Koschorreck, 2023).

Overall, our results indicate that $N_2O$ emissions from aquaculture ponds are primarily constrained by nitrogen substrate availability, sediment redox conditions, and organic carbon supply, rather than by nitrogen enrichment alone. Consequently, $N_2O$ acts as a minor and highly variable component of greenhouse gas exchange in these systems, frequently shifting between weak source and sink behavior.

*$N_2$ Emissions*

$N_2$ fluxes exhibited distinct emission patterns in the aquaculture ponds. Diffusive fluxes dominated $N_2$ exchange. Ebullitive fluxes showed no significant variation with time or treatment. Although dissolved $N_2$ was not measured, the predominance of diffusive fluxes indicates that $N_2$ supersaturation in the water column was likely below the level required to promote bubble-mediated release. Sikar et al. (2019) demonstrated that ebullitive and diffusive $N_2$ fluxes tend to occur at similar rates only when dissolved nitrogen concentrations reach approximately 1.96–3.36 mg N $L^{-1}$. In the present study, total nitrogen concentrations in the water

column (1.02–1.32 mg $L^{-1}$) remained below this transition range, which explains the dominance of diffusive exchange. In contrast, Flickinger et al. (2019) reported higher nitrogen concentrations (~2.6 mg $L^{-1}$), falling within this transition zone, which may account for the prevalence of ebullitive $N_2$ fluxes observed in their study. A similar dominance of diffusive $N_2$ exchange was reported by Higgins et al. (2008). Although nitrogen concentrations were not explicitly discussed by the authors, dissolved organic carbon values in their study (5–7 mg $L^{-1}$) suggest, based on the typical C:N ratio of approximately 10:1 in aquatic systems (Boyd, 1995), relatively low nitrogen availability, which may likewise have limited $N_2$ supersaturation and favored diffusive release. Taken together, these findings suggest that water-column nitrogen availability strongly modulates the relative importance of diffusive versus ebullitive $N_2$ fluxes in shallow aquaculture ponds.

In aquaculture ponds, organic matter derived from feed and feces accumulates in sediments and supports anaerobic nitrogen transformations (Silva et al., 2018). Consistent with this context, our results showed a positive correlation between total $N_2$ fluxes and sediment phosphorus, indicating that nutrient enrichment, particularly phosphorus, stimulates microbial activity and denitrification processes. In contrast, the negative relationship between diffusive $N_2$ fluxes and sediment nitrogen indicates that nitrogen accumulation alone does not necessarily translate into greater $N_2$ release. A similar pattern was reported by Higgins et al. (2008), who showed that $N_2$ release in rivers was mainly controlled by temperature and dissolved organic carbon, while nitrate availability did not limit denitrification, reinforcing that nitrogen concentration alone is not a primary driver of $N_2$ emissions. The negative correlation between ebullitive $N_2$ fluxes and total dissolved solids suggests that ebullitive $N_2$ fluxes were not directly linked to changes in dissolved constituents of the water column. Overall, the weak and sometimes contrasting relationships between $N_2$ fluxes and sediment or water variables are consistent with previous findings showing that $N_2$ production is not directly governed by bulk concentrations of carbon, nitrogen, or phosphorus, but rather by complex microbial and redox controls (Castine et al., 2012; Higgins et al., 2008).

The predominance of $N_2$ fluxes over $N_2O$ emissions indicates that denitrification in these ponds likely proceeded to completion, favoring the reduction of $N_2O$ to $N_2$ (Barreto et al., 2024; Castine et al., 2012; Yuan et al., 2019). This interpretation is consistent with the low and often negative $N_2O$ fluxes observed in the present study and supports the view that aquaculture ponds may function as effective nitrogen removal systems rather than as important sources of reactive nitrogen gases.

*$CO_2$ eq. Emissions*

Total $CO_2$-equivalent emissions varied significantly over the production cycle, increasing toward mid- and late stages. This pattern was driven primarily by ebullitive $CH_4$ emissions, while diffusive $CH_4$ and $N_2O$ fluxes remained stable, indicating that $CO_2$-eq dynamics were governed mainly by methane emissions. Negative correlations with sediment carbon, nitrogen, and phosphorus suggest that higher nutrient accumulation did not directly enhance greenhouse gas release, reflecting the dissociating between sediment production potential and actual emissions. Similar patterns were reported by Vroom et al. (2023), who showed that sediment $CH_4$ production potential did not correlate with in situ emissions, emphasizing the role of surface-water conditions and biological disturbance. Overall, these findings highlight the dominant influence of methane ebullition and in situ biogeochemical controls on the climatic footprint of aquaculture ponds, reinforcing the need for separate quantification of diffusive and ebullitive fluxes, temporally intensive monitoring for accurate greenhouse gas assessments.

In the present study, $CO_2$-equivalent ($CO_2$-eq) emissions per kilogram of fish were calculated solely from direct greenhouse gas fluxes at the water–atmosphere interface of the ponds, excluding other sources typically considered in life-cycle assessments, such as feed production, energy use, or land-use change. Average emission intensities observed were 0.61 ± 0.24 kg $CO_2$-eq $kg^{-1}$ in monoculture (T) and 0.72 ± 0.17 kg $CO_2$-eq $kg^{-1}$ in integrated ponds (TC), with no significant difference between treatments. Direct pond emissions generally account for only 15–18% of the total $CO_2$-eq footprint (Lima et al., 2026; Medeiros et al., 2017). Lima et al. (2026) reported total emissions of 3.9 and 4.3 kg $CO_2$-eq $kg^{-1}$ fish for tambaqui monoculture and integrated systems, respectively, of which 15–17% corresponded to direct rearing emissions (≈0.64–0.66 kg $CO_2$-eq $kg^{-1}$), similar to our findings despite the authors estimated diffusive fluxes. Medeiros et al. (2017) reported lower direct emissions (0.48 kg $CO_2$-eq $kg^{-1}$) in tambaqui monoculture, likely reflecting the production of smaller fish, consistent with literature showing higher emissions at later growth stages (Yuan et al., 2021). These results highlight that direct in situ emissions represent only a small fraction of aquaculture's overall carbon footprint.

## Conclusion

The integration of curimba into tambaqui monoculture did not significantly affect $CH_4$, $N_2O$, or $N_2$ fluxes. However, integrated ponds acted predominantly as net $CO_2$ sources, whereas monoculture ponds functioned mainly as $CO_2$ sinks, indicating that the inclusion of a benthic species modified internal carbon dynamics without intensifying the system's greenhouse gas footprint, highlighting a

dissociating between productivity gains and greenhouse gas impacts. Methane emissions were dominated by ebullition, whereas $CO_2$, $N_2$, and $N_2O$ were released almost exclusively through diffusion, underscoring the importance of distinguishing emission pathways. Although $CH_4$ emissions were not affected by the production system, ebullitive fluxes increased over the production cycle and were primarily associated with eutrophication-related variables, highlighting the key role of sediment organic matter and anaerobic processes in controlling methane release. $N_2O$ fluxes remained near zero, with ponds alternately acting as minor sources and sinks, indicating a negligible contribution to greenhouse gas emissions. The substantial variability observed among replicate measurements highlights the spatial heterogeneity of gas emissions in aquaculture ponds and emphasizes the importance of adequate replication and temporal monitoring when designing greenhouse gas studies in aquatic production systems. By combining temporal monitoring with separate measurements of diffusive and ebullitive fluxes, this study advances the understanding of greenhouse gas dynamics in tropical aquaculture ponds and shows that benthic species integration can improve production efficiency without increasing greenhouse gas emissions.

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## Supplementary data

Table S1. Pearson correlation coefficients (r) and p-values between environmental variables and diffusive greenhouse gas fluxes. Values are Pearson's correlation coefficients (r) with p-values in parentheses. Significant correlations ($P < 0.05$) are shown in bold.

| Environmental variable | $CO_2$ | | $CH_4$ | | $N_2O$ | | $N_2$ | |
|---|---|---|---|---|---|---|---|---|
| | r | *P* | r | *P* | r | *P* | r | *P* |
| Temperature | **0.36** | **0.02** | −0.01 | 0.97 | 0.19 | 0.25 | 0.01 | 0.97 |
| Dissolved oxygen | **−0.42** | **0.01** | 0.08 | 0.65 | −0.12 | 0.45 | −0.11 | 0.51 |
| pH | −0.06 | 0.71 | −0.02 | 0.88 | −0.19 | 0.26 | −0.23 | 0.17 |
| Conductivity | **0.50** | **0.001** | 0.21 | 0.22 | 0.12 | 0.46 | −0.08 | 0.65 |
| Transparency | −0.30 | 0.06 | −0.07 | 0.67 | −0.08 | 0.63 | −0.07 | 0.66 |
| Alkalinity | **0.55** | **<0.001** | 0.22 | 0.18 | 0.12 | 0.47 | −0.05 | 0.77 |
| Hardness | **0.51** | **<0.001** | 0.18 | 0.28 | 0.14 | 0.39 | −0.19 | 0.26 |
| Chlorophyll-a | −0.12 | 0.46 | −0.05 | 0.74 | 0.07 | 0.65 | 0.05 | 0.76 |
| Total dissolved solids | **0.29** | **0.07** | 0.18 | 0.29 | 0.04 | 0.81 | −0.18 | 0.28 |
| Total suspended solids | 0.14 | 0.39 | −0.17 | 0.30 | 0.14 | 0.41 | 0.06 | 0.72 |
| Turbidity | 0.23 | 0.14 | −0.16 | 0.33 | 0.21 | 0.20 | 0.03 | 0.88 |
| Total inorganic carbon (water) | **0.45** | **0.004** | 0.12 | 0.47 | 0.06 | 0.70 | −0.12 | 0.46 |
| Total carbon (water) | **0.41** | **0.009** | 0.10 | 0.56 | 0.04 | 0.81 | −0.02 | 0.89 |
| Total organic carbon (water) | 0.23 | 0.15 | 0.04 | 0.80 | 0.00 | 0.99 | 0.13 | 0.45 |
| Total phosphorus (water) | 0.18 | 0.30 | 0.18 | 0.31 | −0.02 | 0.91 | 0.27 | 0.13 |
| Total nitrogen (water) | 0.04 | 0.80 | 0.01 | 0.93 | 0.11 | 0.52 | 0.01 | 0.95 |
| Sediment nitrogen | 0.17 | 0.38 | 0.15 | 0.46 | 0.25 | 0.22 | **−0.43** | **0.031** |
| Sediment carbon | 0.07 | 0.72 | 0.13 | 0.53 | 0.12 | 0.55 | −0.36 | 0.08 |
| Sediment phosphorus | 0.31 | 0.12 | 0.15 | 0.46 | 0.35 | 0.08 | −0.34 | 0.09 |

Table S2. Pearson correlation coefficients (r) and p-values between environmental variables and ebullitive greenhouse gas fluxes. Values are Pearson's correlation coefficients (r) with p-values in parentheses. Significant correlations ($P < 0.05$) are shown in bold.

| Environmental variable | $CO_2$ | | $CH_4$ | | $N_2O$ | | $N_2$ | |
|---|---|---|---|---|---|---|---|---|
| | r | *P* | r | *P* | r | *P* | r | *P* |
| Temperature | 0.28 | 0.09 | 0.21 | 0.19 | 0.12 | 0.46 | −0.30 | 0.06 |
| Dissolved oxygen | **−0.32** | **0.04** | −0.10 | 0.56 | −0.02 | 0.92 | 0.29 | 0.07 |
| pH | −0.24 | 0.15 | −0.05 | 0.79 | 0.27 | 0.10 | 0.07 | 0.68 |
| Conductivity | 0.02 | 0.90 | 0.00 | 0.99 | −0.19 | 0.25 | −0.08 | 0.65 |
| Transparency | −0.29 | 0.07 | −0.21 | 0.19 | 0.10 | 0.53 | −0.12 | 0.47 |
| Alkalinity | 0.16 | 0.33 | 0.13 | 0.43 | −0.06 | 0.73 | −0.06 | 0.73 |
| Hardness | 0.01 | 0.93 | 0.06 | 0.72 | −0.01 | 0.96 | −0.14 | 0.39 |
| Chlorophyll-a | 0.26 | 0.11 | **0.35** | **0.028** | −0.00 | 0.99 | 0.20 | 0.22 |
| Total dissolved solids | −0.25 | 0.13 | −0.22 | 0.18 | −0.26 | 0.11 | **−0.38** | **0.017** |
| Total suspended solids | **0.41** | **0.010** | **0.50** | **0.001** | 0.16 | 0.32 | 0.29 | 0.07 |
| Turbidity | **0.36** | **0.024** | **0.35** | **0.03** | 0.07 | 0.69 | 0.18 | 0.27 |
| Total inorganic carbon (water) | −0.01 | 0.94 | −0.02 | 0.88 | −0.24 | 0.15 | −0.24 | 0.14 |
| Total carbon (water) | 0.11 | 0.51 | 0.13 | 0.44 | −0.19 | 0.24 | −0.15 | 0.35 |
| Total organic carbon (water) | 0.24 | 0.14 | 0.29 | 0.07 | −0.06 | 0.70 | 0.01 | 0.93 |
| Total phosphorus (water) | 0.13 | 0.47 | **0.56** | **<0.001** | −0.03 | 0.85 | 0.11 | 0.54 |
| Total nitrogen (water) | −0.06 | 0.69 | 0.01 | 0.97 | −0.02 | 0.90 | −0.03 | 0.85 |
| Sediment nitrogen | 0.06 | 0.78 | −0.14 | 0.49 | 0.10 | 0.61 | −0.04 | 0.84 |
| Sediment carbon | −0.13 | 0.51 | −0.14 | 0.49 | 0.17 | 0.40 | 0.12 | 0.54 |
| Sediment phosphorus | 0.21 | 0.30 | 0.07 | 0.74 | 0.36 | 0.06 | 0.29 | 0.14 |

**Table S3.** Pearson correlation coefficients (r) and p-values between environmental variables and total greenhouse gas fluxes and $CO_2$-equivalent emissions. Values are Pearson's correlation coefficients (r) with p-values in parentheses. Significant correlations ($P < 0.05$) are shown in bold.

| Environmental variable | $CO_2$ | | $CH_4$ | | $N_2O$ | | $N_2$ | | $CO_2$eq | |
|---|---|---|---|---|---|---|---|---|---|---|
| | r | *P* | r | *P* | r | *P* | r | *P* | r | *P* |
| Temperature | **0.45** | **0.01** | 0.11 | 0.52 | 0.06 | 0.75 | 0.09 | 0.62 | 0.08 | 0.68 |
| Dissolved oxygen | **−0.44** | **0.01** | 0.07 | 0.70 | −0.11 | 0.53 | −0.13 | 0.45 | −0.10 | 0.59 |
| pH | −0.07 | 0.69 | −0.01 | 0.96 | −0.26 | 0.13 | −0.16 | 0.34 | −0.23 | 0.21 |
| Conductivity | **0.56** | **<0.001** | 0.00 | 0.98 | −0.06 | 0.74 | 0.03 | 0.87 | −0.11 | 0.57 |
| Transparency | −0.31 | 0.06 | −0.14 | 0.41 | −0.10 | 0.58 | −0.05 | 0.76 | 0.00 | 0.98 |
| Chlorophyll-a | −0.12 | 0.49 | 0.30 | 0.08 | 0.07 | 0.71 | 0.03 | 0.86 | 0.08 | 0.67 |
| Total dissolved solids | **0.35** | **0.03** | −0.14 | 0.42 | −0.22 | 0.21 | 0.04 | 0.84 | −0.22 | 0.25 |
| Total suspended solids | 0.15 | 0.39 | 0.29 | 0.09 | 0.08 | 0.65 | 0.09 | 0.60 | 0.01 | 0.96 |
| Turbidity | 0.25 | 0.13 | 0.17 | 0.32 | 0.05 | 0.79 | 0.15 | 0.37 | −0.03 | 0.88 |
| Alkalinity | **0.60** | **<0.001** | 0.12 | 0.51 | −0.05 | 0.78 | 0.10 | 0.55 | −0.12 | 0.52 |
| Hardness | **0.58** | **<0.001** | 0.01 | 0.97 | −0.17 | 0.32 | 0.05 | 0.76 | −0.26 | 0.16 |
| Total inorganic carbon (water) | **0.51** | **0.001** | −0.00 | 0.99 | −0.14 | 0.43 | 0.02 | 0.91 | −0.10 | 0.61 |
| Total carbon (water) | **0.47** | **0.003** | 0.12 | 0.48 | −0.02 | 0.91 | −0.04 | 0.83 | 0.04 | 0.85 |
| Total organic carbon (water) | 0.26 | 0.11 | 0.25 | 0.15 | 0.15 | 0.39 | −0.09 | 0.60 | 0.20 | 0.28 |
| Total phosphorus (water) | 0.19 | 0.32 | **0.47** | **0.011** | 0.29 | 0.12 | −0.08 | 0.67 | 0.23 | 0.25 |
| Total nitrogen (water) | 0.07 | 0.68 | 0.04 | 0.81 | 0.01 | 0.94 | 0.04 | 0.80 | 0.08 | 0.66 |
| Sediment nitrogen | 0.19 | 0.37 | −0.05 | 0.81 | **−0.43** | **0.042** | 0.25 | 0.24 | **−0.54** | **0.018** |
| Sediment carbon | 0.04 | 0.84 | −0.04 | 0.85 | −0.37 | 0.086 | 0.20 | 0.35 | **−0.46** | **0.048** |
| Sediment phosphorus | 0.30 | 0.14 | 0.05 | 0.82 | −0.34 | 0.11 | **0.43** | **0.035** | **−0.60** | **0.007** |

**Table S4.** The mean ± standard deviation of the water quality parameters measured in tambaqui (*Colossoma macropomum*) monoculture (T) and integrated culture of tambaqui and curimba (*Prochilodus lineatus*) (TC) in ponds. Different letters in the same row indicate significant differences (Tukey test, $P < 0.05$). Treat – Treatment.

| Variables | Treatments | | *P* value | | |
|---|---|---|---|---|---|
| | T | TC | Treatment | Time | Treat*Time |
| *Water variables* | | | | | |
| Temperature (°C) | 29 ± 1.8 | 28.9 ± 1.6 | 0.5486 | <0.0001 | 0.682 |
| Dissolved oxygen (mg $L^{-1}$) | **6.0 ± 1.7 a** | **5.5 ± 1.6 b** | 0.0280 | 0.0011 | 0.0890 |
| Conductivity (µ S $cm^{-1}$) | 92.4 ± 29.8 | 110.4 ± 24.8 | 0.2482 | 0.0001 | 0.9868 |
| pH | 8.0 ± 0.7 | 7.9 ± 0.7 | 0.2384 | <0.0001 | 0.0312 |
| Alkalinity (mg L-1) | 41.9 ± 12.2 | 48.5 ± 10.0 | 0.1124 | 0.2388 | 0.9812 |
| Total Nitrogen (mg L-1) | **1.02 ± 0.57 b** | **1.32 ± 0.62 a** | 0.0838 | 0.2162 | 0.2292 |
| Total Phosphorus (µg L-1) | 83.5 ± 44.5 | 93.5 ± 56.1 | 0.5123 | <0.0001 | 0.3534 |
| Total Dissolved Phosphorus (µg L-1) | 32.5 ± 19.8 | 32.8 ± 18.3 | 0.8675 | <0.0001 | 0.4562 |
| Total inorganic carbon (mg L-1) | 6.38 ± 1.42 | 6.82 ± 1.63 | 0.1164 | <.0001 | 0.1003 |
| Total organic carbon (mg L-1) | 3.96 ± 1.58 | 4.42 ± 1.71 | 0.2693 | <.0001 | 0.6049 |
| Total carbon (mg L-1) | 10.34 ± 2.79 | 11.24 ± 3.18 | 0.1227 | <.0001 | 0.3242 |
| Chlorophyll-a (mg L-1) | **19 ± 27.4 b** | **34.5 ± 37.2 a** | 0.0442 | 0.8384 | 0.1592 |
| Total Dissolved Solids (mg L-1) | **86.6 ± 41 b** | **104.7 ± 55.6 a** | 0.0216 | <0.0001 | 0.4271 |
| Total Suspended Solids (mg L-1) | **11.8 ± 10.9 b** | **25.6 ± 24.7 a** | 0.0117 | 0.0001 | 0.4417 |
| Turbidity (NTU) | **28.5 ± 29.3 b** | **49 ± 41.1 a** | 0.0022 | 0.0482 | 0.7848 |
| *Sediment variables* | | | | | |
| Sediment nitrogen (%) | **0.07 ±0.02 b** | **0.08 ± 0.02 a** | 0.0097 | 0.0971 | 0.1528 |
| Sediment carbon (%) | 0.86 ± 0.23 | 0.92 ± 0.19 | 0.3084 | 0.1302 | 0.4239 |
| Sediment phosphorus (g/kg) | **1.23 ±0.16 b** | **1.38 ± 0.19 a** | 0.0440 | 0.5658 | 0.5720 |

**Table S5.** Diffusive Fluxes (mg $m^{-2}$ $day^{-1}$) of $CO_2$, $CH_4$, $N_2O$, and $N_2$ from ponds under *Colossoma macropomum* monoculture (T) and integrated culture with curimba *Prochilodus lineatus* (TC). Values reported with a standard deviation of ±0.0 represent a single measurement for that specific treatment and month, due to the loss or exclusion of other experimental replicates.

| Treatment | Month | Diffusive fluxes (mg $m^{-2}$ $day^{-1}$) | | | |
|---|---|---|---|---|---|
| | | $CO_2$ | $CH_4$ | $N_2O$ | $N_2$ |
| T | 0 | -3328.5 ± 4203.3 | 2.55 ± 9.01 | -0.3497 ± 0.3334 | 5038.3 ± 12840.5 |
| | 2 | -2313.8 ± 2272.7 | 40.39 ± 45.11 | -0.0595 ± 0.1857 | 6014.9 ± 32143.7 |
| | 3 | -471.6 ± 556.7 | 0.51 ± 42.43 | -0.2397 ± 0.3331 | 19355.4 ± 12156.3 |
| | 4 | -1753.4 ± 0.0 | 29.49 ± 1.75 | 0.0351 ± 0.6448 | 31092.7 ± 44476.5 |
| | 5 | 1636.7 ± 2254.7 | -25.67 ± 31.50 | 0.0366 ± 0.0544 | -14986.6 ± 43347.5 |
| | 6 | -744.5 ± 597.4 | 28.91 ± 23.68 | 0.3110 ± 0.2881 | -2518.7 ± 15209.8 |
| | 7 | -786.1 ± 1275.0 | 9.78 ± 8.38 | 0.0695 ± 0.1905 | 10467.2 ± 25483.4 |
| TC | 0 | -745.6 ± 585.3 | 15.52 ± 7.36 | -0.0174 ± 0.1843 | 3268.9 ± 16818.6 |
| | 2 | 1868.9 ± 1636.8 | 12.99 ± 8.99 | 0.0228 ± 0.3205 | -6953.7 ± 7225.4 |
| | 3 | 1001.5 ± 1120.2 | 27.57 ± 47.19 | 0.4132 ± 0.4769 | -29257.8 ± 22824.1 |
| | 4 | 1787.0 ± 3992.8 | 35.30 ± 16.81 | -0.1036 ± 0.1728 | 2080.2 ± 0.0 |
| | 5 | 1157.2 ± 1368.7 | 8.50 ± 35.05 | -0.1301 ± 0.5198 | 33906.8 ± 36114.1 |
| | 6 | -244.8 ± 1587.8 | 19.97 ± 19.92 | 0.5296 ± 0.3862 | -12367.5 ± 7403.0 |
| | 7 | -2607.6 ± 693.6 | 8.78 ± 17.90 | 0.1385 ± 0.4555 | 39099.1 ± 12670.5 |

**Table S6.** Ebullitive Fluxes (mg $m^{-2}$ $day^{-1}$) of $CO_2$, $CH_4$, $N_2O$, and $N_2$ from ponds under *Colossoma macropomum* monoculture (T) and integrated culture with curimba *Prochilodus lineatus* (TC). Values reported with a standard deviation of ±0.0 represent a single measurement for that specific treatment and month, due to the loss or exclusion of other experimental replicates.

| Treatment | Month | Ebullitive fluxes (mg $m^{-2}$ $day^{-1}$) | | | |
|---|---|---|---|---|---|
| | | $CO_2$ | $CH_4$ | $N_2O$ | $N_2$ |
| T | 0 | 0.46 ± 0.33 | 7.18 ± 9.93 | 0.0002 ± 0.0003 | 273.7 ± 343.3 |
| | 2 | 1.03 ± 1.53 | 41.61 ± 69.94 | 0.0002 ± 0.0003 | 218.5 ± 317.5 |
| | 3 | 0.24 ± 0.10 | 7.41 ± 4.74 | 0.0001 ± 0.0000 | 89.2 ± 17.6 |
| | 4 | 1.98 ± 1.77 | 104.73 ± 90.82 | 0.0004 ± 0.0005 | 204.7 ± 167.7 |
| | 5 | 3.68 ± 2.13 | 20.11 ± 32.80 | 0.0015 ± 0.0021 | 151.3 ± 168.5 |
| | 6 | 1.82 ± 0.29 | 52.91 ± 46.57 | 0.0003 ± 0.0003 | 221.7 ± 156.2 |
| | 7 | 1.32 ± 0.71 | 14.95 ± 14.03 | 0.0003 ± 0.0002 | 261.8 ± 149.0 |
| TC | 0 | 0.34 ± 0.14 | 9.63 ± 6.27 | 0.0003 ± 0.0002 | 341.6 ± 205.0 |
| | 2 | 0.95 ± 0.89 | 5.64 ± 9.16 | 0.0001 ± 0.0000 | 70.0 ± 39.8 |
| | 3 | 2.27 ± 3.72 | 5.56 ± 9.36 | 0.0000 ± 0.0000 | 68.9 ± 70.6 |
| | 4 | 1.89 ± 1.10 | 48.96 ± 80.92 | 0.0001 ± 0.0001 | 183.5 ± 181.1 |
| | 5 | 6.63 ± 3.82 | 60.02 ± 48.18 | 0.0004 ± 0.0005 | 184.8 ± 49.3 |
| | 6 | 4.55 ± 2.00 | 90.10 ± 64.85 | 0.0011 ± 0.0009 | 562.4 ± 195.9 |
| | 7 | 4.90 ± 3.52 | 34.69 ± 19.40 | 0.0004 ± 0.0004 | 394.5 ± 250.9 |

**Table S7.** Total Fluxes (mg $m^{-2}$ $day^{-1}$) of $CO_2$, $CH_4$, $N_2O$, $N_2$, and $CO_2$ eq. from ponds under *Colossoma macropomum* monoculture (T) and integrated system with curimba *Prochilodus lineatus* (TC). Values reported with a standard deviation of ±0.0 represent a single measurement for that specific treatment and month, due to the loss or exclusion of other experimental replicates.

| Treatment | Month | Total fluxes (mg $m^{-2}$ $day^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | $CO_2$ | $CH_4$ | $N_2O$ | $N_2$ | $CO_2$ eq. |
| T | 0 | -3328.2 ± 4203.0 | 9.65 ± 19.05 | -0.3495 ± 0.3335 | 5220.8 ± 12649.5 | 138.6 ± 626.4 |
| | 2 | -2312.8 ± 2273.0 | 82.00 ± 113.75 | -0.0593 ± 0.1860 | 6233.4 ± 32417.0 | 2214.2 ± 3142.5 |
| | 3 | -471.4 ± 556.6 | 5.45 ± 37.76 | -0.2397 ± 0.3331 | 19414.9 ± 12185.8 | 82.9 ± 936.5 |
| | 4 | -1752.8 ± 0.0 | 105.49 ± 105.69 | 0.0352 ± 0.6448 | 31297.4 ± 44640.1 | 2878.8 ± 2698.7 |
| | 5 | 1640.3 ± 2252.7 | -5.56 ± 20.33 | 0.0382 ± 0.0565 | -14835.3 ± 43478.9 | -140.9 ± 537.6 |
| | 6 | -742.6 ± 597.6 | 81.82 ± 25.62 | 0.3113 ± 0.2882 | -2296.9 ± 15087.4 | 2310.6 ± 677.3 |
| | 7 | -784.8 ± 1274.5 | 24.74 ± 21.65 | 0.0698 ± 0.1907 | 10729.0 ± 25399.7 | 691.9 ± 550.2 |
| TC | 0 | -745.4 ± 585.5 | 21.94 ± 2.58 | -0.0172 ± 0.1844 | 3496.6 ± 16573.9 | 592.0 ± 47.0 |
| | 2 | 1869.8 ± 1637.7 | 21.42 ± 20.01 | 0.0228 ± 0.3205 | -6895.2 ± 7176.7 | 547.1 ± 474.3 |
| | 3 | 1003.8 ± 1118.0 | 33.13 ± 42.64 | 0.4132 ± 0.4769 | -29188.9 ± 22797.4 | 1013.9 ± 1275.0 |
| | 4 | 1788.9 ± 3992.6 | 84.26 ± 81.03 | -0.1035 ± 0.1728 | 2472.5 ± 0.0 | 2263.6 ± 2187.6 |
| | 5 | 1163.8 ± 1368.1 | 68.51 ± 17.91 | -0.1297 ± 0.5203 | 34091.6 ± 36077.5 | 1828.2 ± 509.8 |
| | 6 | -240.2 ± 1588.6 | 110.07 ± 57.42 | 0.5309 ± 0.3873 | -11915.7 ± 7460.1 | 2319.1 ± 814.4 |
| | 7 | -2602.7 ± 690.8 | 32.92 ± 27.05 | 0.1160 ± 0.3249 | 39493.6 ± 12915.2 | 879.8 ± 784.8 |

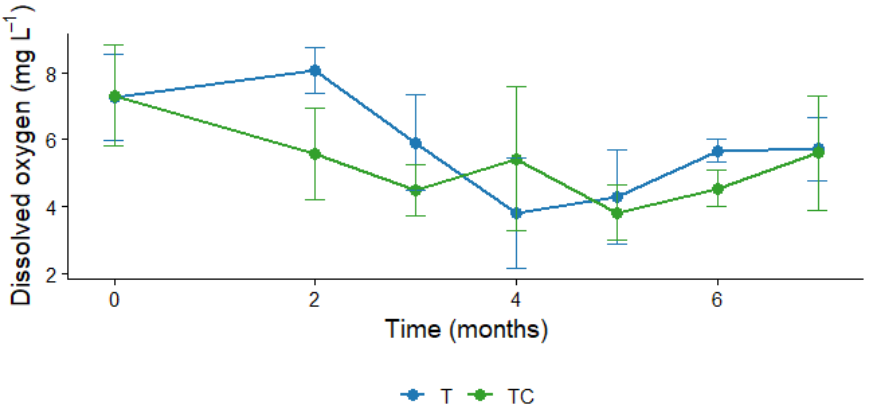


**Figure S1.** Temporal variation of dissolved oxygen (mg $L^{-1}$) in ponds under tambaqui *Colossoma macropomum* monoculture (T) and integrated system with curimba *Prochilodus lineatus* (TC). Values represent means ± standard deviation across experimental units at each sampling time.

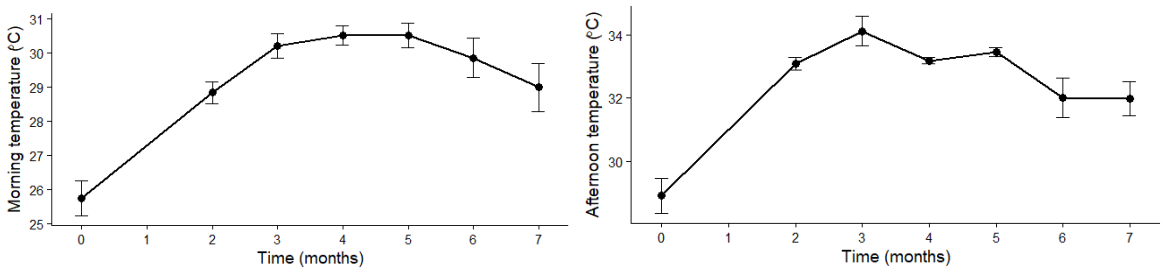


**Figure S2.** Temporal variation of morning and afternoon water temperature in the ponds throughout the experimental period. Values represent means ± standard deviation of all ponds, with data pooled across treatments. Lines indicate temporal trends over time.

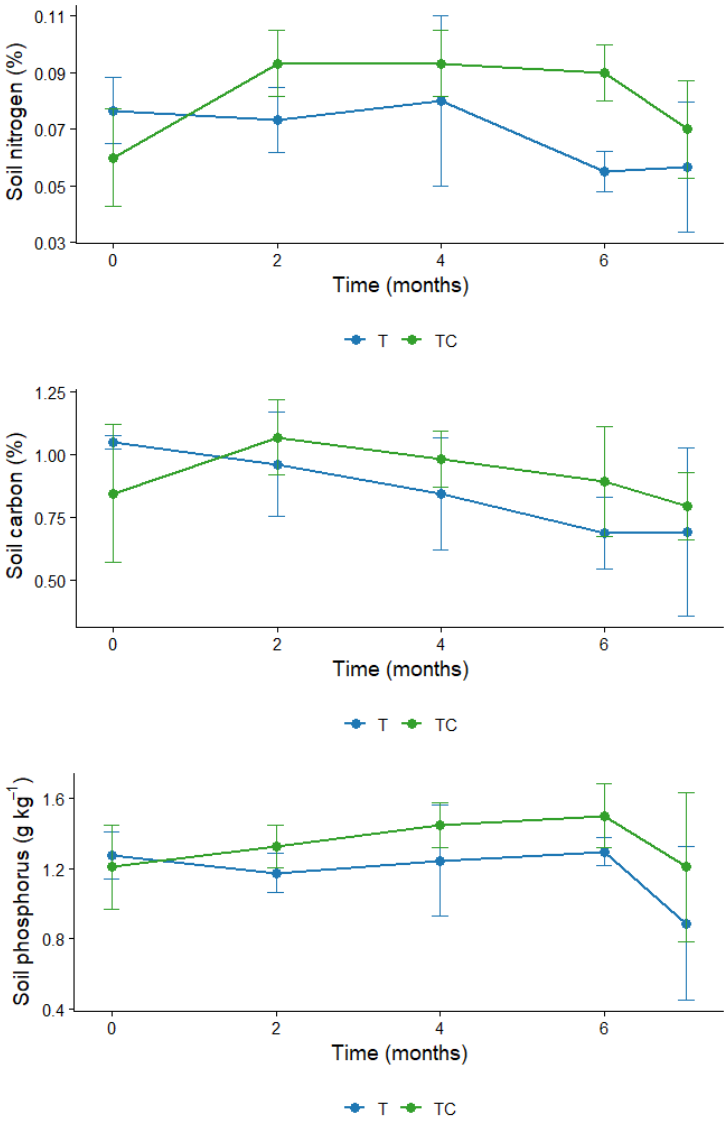


**Figure S3.** Temporal variation of soil nitrogen (%), soil carbon (%), and soil phosphorus (g kg$^{-1}$) under tambaqui *Colossoma macropomum* monoculture (T) and integrated system with curimba *Prochilodus lineatus* (TC). Values represent means ± standard deviation across experimental units at each sampling time.

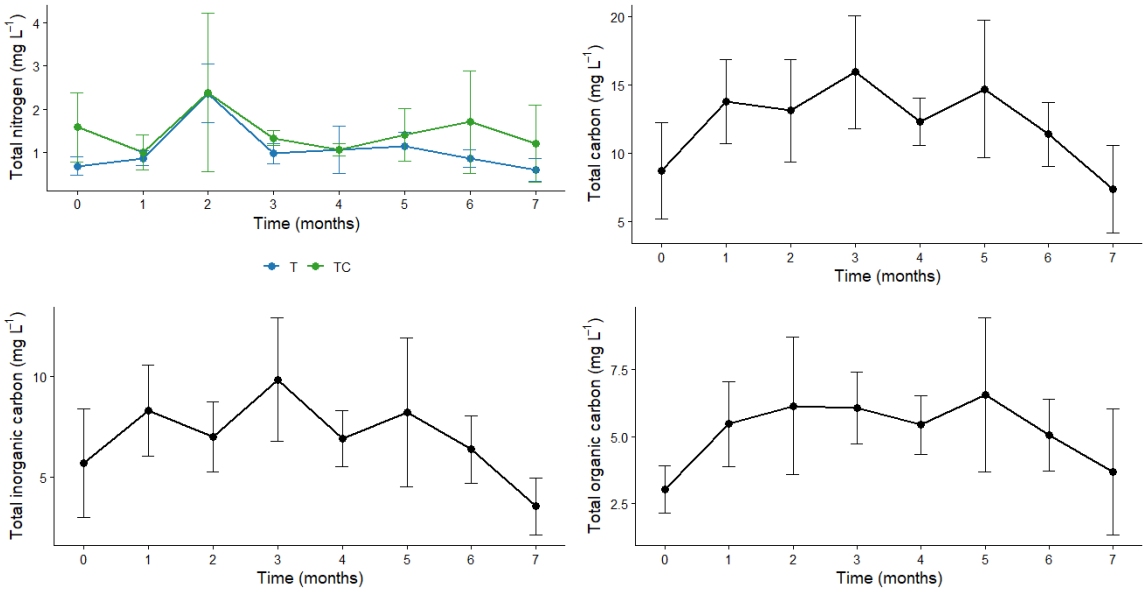


**Figure S4.** Temporal variation of total inorganic carbon (TIC), total carbon (TC), total organic carbon (TOC), and total nitrogen (TN) in water ponds during tambaqui (*Colossoma macropomum*) monoculture (T) and integrated system with curimba (*Prochilodus lineatus*, TC). Values represent means ± standard deviation of replicate ponds. For TIC, TC, and TOC, no significant differences were detected between treatments; therefore, data are presented as pooled means across treatments.

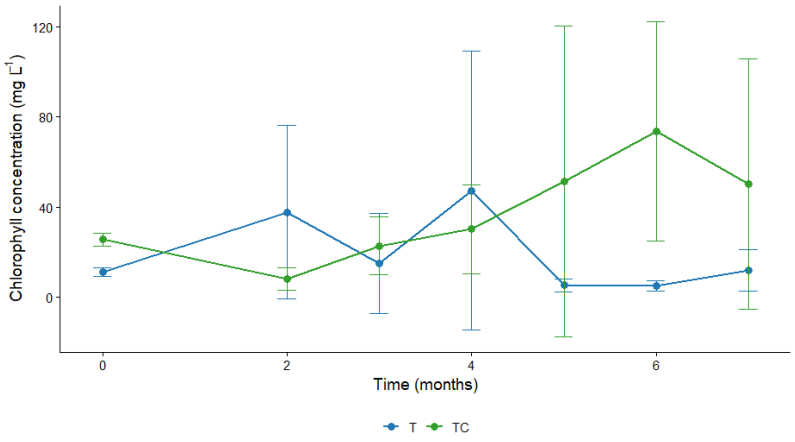


**Figure S5.** Temporal variation of chlorophyll in water pond during tambaqui (*Colossoma macropomum*) monoculture (T) and integrated system with curimba (*Prochilodus lineatus*, TC). Values represent means ± standard deviation of replicate ponds.

## Conclusão geral

A inclusão da curimba na criação de tambaqui não comprometeu o crescimento, a sobrevivência, a produtividade ou a taxa de conversão alimentar da espécie. Essa integração aumentou em 25% a biomassa de peixes e reduziu em 14% a taxa de conversão alimentar, o que evidência melhor eficiência no uso da ração. A presença da curimba no sistema de produção resultou em alterações principalmente nos parâmetros físicos da água, decorrentes da bioturbação do sedimento causada pelo seu comportamento. Essa bioturbação provavelmente liberou nutrientes na coluna d'água, estimulando uma cascata trófica *bottom-up* e permitindo a recuperação desses nutrientes como biota dentro do sistema de viveiro. Além disso, as alterações nos parâmetros da qualidade da água no cultivo integrado possivelmente contribuíram para um aumento na abundância de parasitas. A implementação do cultivo integrado aumentou a receita líquida em US$ 1.481,14 por hectare por ano.

O tambaqui apresentou um padrão consistente de utilização de alimentos quando produzidos com a oferta de ração, enquanto a curimba demonstrou maior plasticidade alimentar. Considerando que o tambaqui é a espécie principal do sistema produtivo, manter sua biomassa em níveis elevados é essencial para minimizar a sobreposição no uso da ração formulada , ao mesmo tempo em que se potencializa a recuperação de nutrientes e a eficiência geral do sistema. A curimba atua como espécie extrativa nos viveiros de cultivo integrado multitrófico, alimentando-se principalmente de zooplâncton (vivo ou morto), contribuindo indiretamente para a redução da matéria orgânica. Um sistema de IMTA com curimba e elevada biomassa de tambaqui promove melhor aproveitamento dos recursos alimentares naturais nos viveiros de produção. O IMTA também aumenta a eficiência ecotrófica tanto da ração quanto do zooplâncton, além de elevar o índice de onivoria do sistema e o índice de ciclagem de Finn.

O cultivo integrado multitrófico de tambaqui e curimba reduziu todas as categorias de impacto ambiental em análises de ciclo de vida, com destaque para a diminuição da eutrofização em água doce e da dependência hídrica. A ração é o

principal fator contribuinte para a demanda energética, mudanças climáticas e produção líquida primária, enquanto a fase de cultivo desempenha papel predominante na acidificação, eutrofização, eutrofização marinha, competição por terra e dependência hídrica. A maior biomassa de peixes produzida no sistema IMTA melhora tanto a taxa de conversão alimentar quanto a recuperação de nutrientes nos viveiros, fatores-chave que explicam a redução do impacto ambiental em comparação ao monocultivo de tambaqui. Assim, a maior eficiência alimentar e a maior recuperação de nutrientes no sistema IMTA são centrais para seu melhor desempenho ambiental.

A inclusão do curimba na produção de tambaqui não alterou os fluxos de $CH_4$, $N_2O$ e $N_2$ dos viveiros de produção. Entretanto, o cultivo integrado atuou emitindo $CO_2$, enquanto o monocultivo absorveu $CO_2$, indicando modificações na dinâmica interna do carbono. Essa mudança não resulta em aumento de impactos ambientais, dado que o $CO_2$ emitido pela aquicultura é de origem biogênica e, portanto, não é contabilizado nos inventários de aquecimento global da aquicultura. As emissões de $CH_4$ foram dominadas pela via ebulitiva, que se intensificou ao longo do ciclo produtivo e esteve associada a variáveis relacionadas à eutrofização, evidenciando a importância do sedimento na regulação do metano. O $N_2O$ apresentou fluxos próximos de zero, com contribuição mínima para as emissões de gases de efeito estufa. De forma geral, os resultados demonstram que a integração de uma espécie bentônica pode aumentar a eficiência produtiva sem intensificar as emissões de gases de efeito estufa, contribuindo para a sustentabilidade da aquicultura tropical.

Nesse contexto, conclui-se que o tambaqui pode ser cultivado com sucesso em sistemas multitróficos integrados com espécies bentônicas, aumentando a eficiência da produção aquícola e alinhando-se aos princípios da economia circular. A inclusão da curimba aumenta a biomassa total de peixes nos viveiros sem necessidade de insumos adicionais de ração. Isso não apenas melhora a renda dos produtores e o retorno econômico potencial da produção de tambaqui, como também eleva a eficiência na recuperação de nutrientes, consolidando a integração

dessa espécie como uma estratégia promissora para aumentar a circularidade dos sistemas aquícolas. Esses resultados evidenciam ainda que a IMTA, com espécies adequadamente selecionadas, representa uma alternativa mais sustentável aos sistemas convencionais de monocultivo na aquicultura.

Estudos futuros devem explorar outras proporções de tambaqui e curimba, bem como as densidades de estocagem de cada espécie no sistema integrado, a fim de otimizar o crescimento da curimba e a produtividade total. O Brasil, por ser um país com uma grande diversidade biológica, oferece grande potencial para o uso de diferentes combinações de espécies, contribuindo para o avanço da aquicultura integrada.

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