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Tolerância e o potencial papel facilitador de espécies lenhosas em rejeitos de minério de lítio

Maria Gracielle Rodrigues Maciel
Doctor Scientiae

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Thesis submitted to the Ecology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Joao Paulo de Souza

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Dedico aos meus pais e irmãs, minha Fortaleza.

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“Não é o mais forte que sobrevive, nem o mais inteligente, mas o que melhor se adapta às mudanças.” (Charles Darwin)

ABSTRACT

MACIEL, Maria Gracielle Rodrigues, D.Sc., Universidade Federal de Viçosa, November, 2025. **Tolerance and the potential facilitating role of woody species in lithium ore tailings**. Adviser: Joao Paulo de Souza.

Global lithium exploration has grown in recent years, mainly due to its application in technological items, such as batteries. In Brazil, this mineral has significant reserves, mainly in the north of Minas Gerais. Although extraction promotes regional socioeconomic advances, little is known about its damage to the flora and how to mitigate it. Thus, the objective of this study was to evaluate the development and tolerance of four woody species to lithium ore tailings (LOT), as well as their potential for phytoremediation. We studied two species with wide geographic distribution (*Enterolobium contortisiliquum* and *Handroanthus impetiginosus*) and two with a restricted distribution (*Hymenaea courbaril* and *Hymenaea stigonocarpa*). In addition, it was analyzed how different levels of interaction (individual, intra, and interspecific levels) may facilitate the growth of *E. contortisiliquum* and its ability to absorb metals. Plants were grown in LOT and reference soil (RS) under full sun conditions for ± 265 days to evaluate photosynthetic efficiency, growth, and mineral nutrition. The results showed that RML is a limiting factor for the development of tree species, mainly affecting vegetative growth and photosynthetic efficiency. When evaluating the isolated growth of different tree species in pots, all species showed photosynthetic adjustments, but persistent LOT stress compromised biomass production, nutrient uptake, and tolerance over time. The comparison between species with different geographic distributions showed that tolerance to LOT is not determined by the range of occurrence (*H. courbaril* and *H. stigonocarpa* showed greater tolerance, while *E. contortisiliquum* was the most sensitive species). Furthermore, *H. courbaril* was the only species showing potential for phytoextraction, as it presented tolerance and significant Li accumulation in the leaves. When investigating whether the interaction of *E. contortisiliquum* with other species could improve its performance, it was found that this association did not compensate for the adverse effects of LOT on photosynthesis and biomass, confirming the tailings as the primary limiting factor. However, interspecific interaction contributed positively to vegetative growth in a less restrictive substrate such as reference soil (RS), while intraspecific interaction intensified competition and reduced biomass. Despite the lack of effect of interactions on LOT, nutrient and metal concentrations under interspecific interaction conditions indicated that *E. contortisiliquum* absorbed more nutrients and reduced metal accumulation. These results suggest that, although interspecific interactions did not result in direct growth and photosynthetic gains, they played an important ecological role by partially mitigating the effects of toxicity. Furthermore, the greater capacity of some species to accumulate Li (*H. courbaril*) reinforces the role of functional diversity in attenuating toxic effects between interacting species. This study highlights the importance of integrating ecological and physiological approaches in revegetating areas impacted by mining tailings. This evidence provides an initial understanding of the influence of LOT on the early development of native woody species, being *H. courbaril* potential species for Li phytoremediation, and *E. contortisiliquum*, a generalist species tolerant to some metals ((Na, Cu, Zn), was not tolerant to LOT. These findings indicate that selecting adapted species and diversified cultivation can strengthen restoration, promote ecological stability, mitigate toxicity, and conserve biodiversity. Thus, the research provides scientific and practical support for more effective and sustainable strategies for ecosystem recovery.

Keywords: Lithium mining; phytoremediation; vegetative morphometry; photosynthetic parameters.

RESUMO

MACIEL, Maria Gracielle Rodrigues, D.Sc., Universidade Federal de Viçosa, novembro de 2025. **Tolerância e o potencial papel facilitador de espécies lenhosas em rejeitos de minério de lítio**. Orientador: Joao Paulo de Souza.

A exploração global de lítio (Li) tem crescido, sobretudo pelo uso em baterias, e o Brasil possui grandes reservas, especialmente no Norte de Minas Gerais. Contudo, apesar dos benefícios socioeconômicos, ainda são pouco conhecidos os impactos da extração sobre a flora e as formas de mitigá-los. Assim, o objetivo deste estudo foi avaliar o desenvolvimento e a tolerância de quatro espécies lenhosas ao rejeito de minério de lítio (RML), bem como seu potencial de fitorremediação. Foram analisadas duas espécies de ampla distribuição geográfica (*Enterolobium contortisiliquum* e *Handroanthus impetiginosus*) e duas de distribuição mais restrita (*Hymenaea courbaril* e *Hymenaea stigonocarpa*). Além de investigar como diferentes níveis de interação (individual, intra e interespecífica) influenciam o crescimento e a absorção de metais por *E. contortisiliquum*. As plantas foram cultivadas em RML e solo comum (SC), sob sol pleno, por ± 265 dias, para avaliar a eficiência fotossintética, o crescimento e a nutrição mineral. OS resultados evidenciaram que o RML limita o desenvolvimento arbóreo, sobretudo o crescimento vegetativo e a eficiência fotossintética. Quando cultivadas individualmente em vasos, as espécies arbóreas apresentaram ajustes fotossintéticos; porém, o estresse contínuo por RML comprometeu a biomassa, a absorção de nutrientes e a tolerância ao longo do tempo. Além disso, a tolerância não esteve relacionada à distribuição geográfica, sendo H. courbaril e H. stigonocarpa as mais tolerantes e *E. contortisiliquum* a mais sensível. Além disso, H. courbaril foi a única espécie a apresentar potencial de fitoextração, aliando tolerância ao acúmulo significativo de Li nas folhas. Ao avaliar a interação de *E. contortisiliquum* com outras espécies, observou-se que essa associação não mitigou os efeitos do RML sobre a fotossíntese e a biomassa, confirmando o rejeito como fator limitante primário. Contudo, em solo comum (SC), a interação interespecífica favoreceu o crescimento vegetativo, enquanto a intraespecífica intensificou a competição e reduziu a biomassa. Apesar da ausência de efeito das interações no RML, a concentração de nutrientes e metais em condições de interação interespecífica indicou que *E. contortisiliquum* absorveu maiores quantidades de nutrientes e reduziu o acúmulo de metais. Esses resultados sugerem que, embora a interação interespecífica não resulte em ganhos diretos de crescimento e de fotossíntese, ela desempenhou um papel ecológico importante ao reduzir parcialmente os efeitos da toxicidade. Além disso, a maior capacidade de algumas espécies de acumular Li (como *H. courbaril*) reforça o papel da diversidade funcional na atenuação dos efeitos tóxicos entre espécies em interação. Este estudo evidencia a importância de integrar abordagens ecológicas e fisiológicas na revegetação de áreas impactadas por rejeitos de mineração. Essas evidências oferecem um entendimento inicial sobre a influência do RML no desenvolvimento inicial de espécies lenhosas nativas, sendo que *H. courbaril* apresenta potencial para fitorremediação de Li e *E. contortisiliquum*, uma espécie generalista e tolerante a metais (Na, Cu, Zn), não se mostrou tolerante ao RML. Esses achados indicam que a seleção de espécies adaptadas, aliada ao cultivo diversificado, pode fortalecer a restauração, promovendo a estabilidade ecológica, a mitigação da toxicidade e a conservação da biodiversidade. Assim, a pesquisa fornece subsídios científicos e práticos para estratégias mais eficazes e sustentáveis de recuperação de ecossistemas.

Palavras-chave: Mineração de lítio; fitorremediação; morfometria vegetativa; parâmetros fotossintéticos.

LIST OF ACRONYMS AND ABBREVIATIONS

ALA – Average Leaf Area
DBE – Days after the Beginning of the Experiment
DRG – Relative Growth Rate of the stem Diameter
Chl *a* – chlorophyll *a*
Chl *b* – chlorophyll *b*
Chl *total* – chlorophyll *total*
ETR – Electron Transport Rate
Fm – Maximum Fluorescence
F0 – Minimum fluorescence
F_v/F_m – Maximum quantum yield of PSII
HRG – Relative Growth Rate of the stem Height
LAR – Leaf Area Ratio
LA – Leaf Area
LDM – Leaf Dry Mass
LEI – Leaf Expansion Interval
LER – Leaf Expansion Rate
LRG – Relative Growth Rate of the number of Leaves
LLS – Leaf Life Span
LOT – Lithium Ore Tailings
LTI – Leaf Tolerance Index
LN - Leaf Number
NPQ – non-photochemical quenching
OC – Open Chamber
PAR – Photosynthetically Active Radiation
ϕPSII: effective quantum yield of PSII
RDM – Root Dry Mass
RGR – Relative Growth Rate
RII - Relative Interaction Index
RL – Root Length
RML – Rejeito de Minério de Lítio (in Portuguese)
RS – Reference Soil
RSR – Root/Shoot Ratio

RTI – Root Tolerance Index

SD – Stem Diameter

SDM – Stem Dry Mass

SLA – Specific Leaf Area

SL – Stem Length

SC – Solo Comum (in Portuguese)

STI – Stem Tolerance Index

TDM – Total Dry Mass

TF – Translocation Factor

TI – Tolerance Index

TLA – Total Leaf Area

TTI – Total Tolerance Index

SUMÁRIO

INTRODUÇÃO GERAL

Entre os quarenta elementos mais abundantes na natureza, o lítio (Li) encontra-se naturalmente distribuído em pequenas quantidades em rochas, solos e na água. A ocorrência de minerais de Li na natureza ocorre principalmente por meio de evaporitos (salmouras de Li) e rochas ígneas como pegmatitos (Aral; Vecchio-Sadus 2011). Um dos minerais pegmatíticos é o espodumênio, considerado a principal fonte de Li (Roy et al., 2023).

O Li é um dos metais alcalinos mais utilizados pelas indústrias devido às suas diversas aplicações, como armazenamento de energia portátil (baterias), na produção de cerâmicas, vidros, graxas e borrachas (Meshram et al., 2014; Hao et al., 2017; Martin et al., 2017). Dessa forma, a demanda e a exploração global de Li têm aumentado significativamente nos últimos anos, atingindo cerca de 175.000 toneladas em 2015 com estimativas de aumento de cinco vezes para 2029 (Siljkovic et al., 2017; Martin et al., 2017). A busca incessante por Li está levando a um risco global de poluição por esse metal proveniente da fabricação, uso e descarte de produtos que contêm Li (Behr et al., 2023), ocasionando o aumento da sua biodisponibilidade no ambiente, impactando negativamente a biodiversidade dos ecossistemas e também a saúde humana (Elektorowicz, 2015).

No Brasil, as reservas de Li estão associadas a depósitos de pegmatito localizados nos estados de Minas Gerais, Ceará, Rio Grande do Norte e Paraíba. A principal reserva em atividade está localizada no norte de Minas Gerais, no Vale do Jequitinhonha, especificamente entre os municípios de Araçuaí e Itinga (Braga e França, 2013). A extração do Li é realizada pela mineração e, posteriormente, pelo processamento químico em produtos como carbonato e hidróxido de Li (Braga e França, 2013). Em regiões onde a extração de Li ocorre, há uma melhora socioeconômica e, consequentemente, do Índice de Desenvolvimento Humano (IDH). Por outro lado, a intensificação de atividades de mineração e da extração de Li também gera o aumento de depósitos de rejeitos com altas concentrações de elementos químicos, que podem causar grandes problemas de poluição ambiental, afetando os solos, a vegetação e a saúde humana (Aral e Vecchio-Sadus, 2011). O rejeito resultante, identificado como silicato de alumínio ($Al_2O_34SiO_2H_2O$), mantém uma pequena fração de Li mesmo após o processo de extração, permanecendo associado a outros metais (Castro et al., 2016). Esse rejeito é depositado em pilhas ao ar livre, o que pode ocasionar a dispersão de partículas pelas correntes de vento para as áreas do entorno.

Mesmo com um número crescente de estudos sobre a recuperação de ambientes impactados pela mineração (Meyer et al., 2016; Gastauer et al., 2019; Ahirwal e Pandey, 2021; Nunes et al., 2022; Young et al., 2022; Rosado et al., 2023), ainda são escassas as investigações que abordam especificamente os efeitos dos rejeitos da mineração de Li como contaminante ambiental ou as possibilidades de recuperação das áreas afetadas. No Brasil, a maioria dos trabalhos investigam o potencial de processamento das matérias primas (carbonato e hidróxido de Li), crescimento econômico (Braga e França 2013; Martin et al., 2017), a reutilização do rejeito na construção civil (Lemougna et al., 2019), fertilização em espécie de interesse econômico (eucalipto - *Corymbia citriodora* Hook) e barreira física para manejo de plantas daninhas (Castro et al., 2016; Faria, 2017).

Do ponto de vista ambiental, os rejeitos de mineração apresentam características físico-químicas que podem impor severas restrições ao estabelecimento vegetal, como altas concentrações de metais, pH alcalino, granulometria fina e baixa porosidade (Raimondi, 2014; Esteves et al., 2020). O rejeito de minério de Li (RML) possui algumas dessas características (Castro et al., 2016; Maciel et al., 2024), podendo afetar o crescimento das plantas, causando limitações físicas e nutricionais (Nardi et al., 2002; Lier et al., 2010; Esteves et al., 2020; Gagnon et al., 2020). As plantas podem responder de forma positiva ou negativa a diferentes concentrações de metais, isso está relacionado à tolerância de determinadas espécies a esses elementos (Anjum et al., 2016; Roy et al., 2019; Esteves et al., 2020; Melo e Souza, 2025). Por exemplo, apesar do Li não ser considerado um elemento essencial para o desenvolvimento das plantas, em baixas concentrações estimulou o crescimento principalmente das raízes em *Helianthus annuus* L. (Hawrylak-Nowak et al., 2012; Tanveer et al., 2019). No entanto, em concentrações elevadas reduziu o crescimento radicular, o conteúdo de clorofila *a* e *b* e ocasionou necrose foliar em espécies herbáceas (Naranjo et al., 2003; Li et al. 2009; Hawrylak-Nowak et al., 2012).

Os estudos sobre o efeito e a tolerância do Li em espécies arbóreas nativas são escassos (Shahzad et al., 2016; Roy et al., 2019; Kastori et al., 2022; Shakoor et al., 2023[a]; Maciel et al., 2024). A tolerância de espécies arbóreas a determinados níveis de metais pode estar relacionada à amplitude de distribuição geográfica em que ocorrem. De acordo com Meyer et al., (2016), em solos contaminados pelo Cádmio e Zinco, as espécies consideradas de ampla distribuição geográfica apresentaram maior produção de biomassa e acúmulo de metais nas raízes, enquanto aquelas com uma distribuição geográfica mais restrita apresentaram necrose foliar e redução na produção de biomassa aérea.

A recuperação de ambientes degradados por atividades de mineração é um desafio, considerando as condições impostas por esses ambientes como: deficiência nutricional e estrutural do solo, vegetação escassa e alta exposição à radiação (Ettler et al., 2014; Mukhopadhyay et al., 2016; Pourret et al., 2016). Tais condições limitam o estabelecimento de espécies vegetais menos tolerantes (Heckenroth et al., 2016; Ginocchio et al., 2017; Lu et al., 2017). Estudos que consideram o crescimento, produção de biomassa, nutrição mineral e atividades fotossintéticas têm auxiliado na compreensão da tolerância de espécies em ambientes contaminados por metais decorrentes da mineração (Meyer et al., 2016; Esteves et al., 2020; Rosado et al., 2023). A seleção de espécies resistentes pode auxiliar os esforços de fitorremediação em áreas afetadas pela mineração. A fitorremediação é uma técnica ecologicamente correta que utiliza plantas para descontaminar o ambiente, eliminando ou tornando os contaminantes inofensivos, além de ser uma maneira econômica e eficiente de revegetar solos contaminados por metais pesados (Salt et al., 1998; Ashraf et al., 2019; Yan et al., 2020). Portanto, as espécies selecionadas para fitorremediação devem apresentar algumas características específicas, como tolerância e acúmulo de elementos minerais, como os metais pesados, manutenção da atividade fotossintética e da produção de biomassa e plasticidade morfológica (Haridasan et al.,1986; Andrade et al., 2011). A longo prazo, espécies arbóreas têm sido sugeridas para fitorremediação de áreas contaminadas com eficiência superior às herbáceas, especialmente as de crescimento rápido devido à sua capacidade de absorver e armazenar metais, contribuindo para a manutenção dos serviços ecossistêmicos, como o sequestro de carbono e melhorias na qualidade do solo e da água (Gómez et al., 2019).

Tendo em vista que as espécies tolerantes e capazes de bioacumulação são potencialmente fitorremediadoras, melhorando o ambiente, essas espécies também poderiam ser consideradas facilitadoras (Navarro-Cano et al., 2019). A facilitação pode ser entendida como uma interação ecológica que beneficia a entrada, desenvolvimento ou estabelecimento de outras espécies mais sensíveis no ambiente limitante (Navarro-Cano et al., 2019). A facilitação entre espécies vegetais e o uso de espécies com potencial facilitador têm sido propostos como estratégias promissoras para a recuperação ecológica (Brooker et al., 2008; Gómez-Aparicio, 2009), bem como para a manutenção de funções ecossistêmicas em ambientes estressantes, como ambientes degradados pela mineração

(Navarro-Cano et al., 2018; Navarro-Cano et al., 2019). Portanto, o uso simultâneo de diferentes espécies vegetais pode promover a sobrevivência, estabilização e sucessão das plantas durante o processo de reabilitação (Nunes et al., 2020).

Considerando o cenário de impactos ocasionados pela atividade de mineração e depósito de RML, são necessários estudos que visem à mitigação e recuperação de áreas degradadas, especialmente no estado de Minas Gerais. Assim, as respostas encontradas na presente tese nos auxiliarão no: 1) conhecimento dos principais metais associados ao rejeito da mineração de Lítio e suas concentrações no solo, 2) no entendimento sobre a influência desses metais no desenvolvimento de espécies lenhosas e suas estratégias de tolerância e 3) no papel facilitador das interações intraespecífica e interespecífica sobre o estabelecimento e crescimento de espécies vegetais não tolerantes ao RML. Assim, nossos resultados fornecerão subsídios para práticas de recuperação da paisagem no entorno e nas áreas de depósito de rejeito de mineração de Li, visando o reestabelecimento do ecossistema a longo prazo.

A partir disso, nosso objetivo geral foi investigar o efeito do RML em quatro espécies lenhosas com diferentes faixas de distribuição geográfica quanto à tolerância e ao potencial de fitorremediação. Em adição, analisamos como as interações intraespecíficas e interespecíficas podem facilitar o desenvolvimento das plantas, sua capacidade de absorver metais e o estabelecimento de espécies não tolerantes ao RML. Para isso, esta tese foi organizada em dois capítulos, escritos e estruturados como manuscritos para serem enviados a periódicos científicos. O capítulo I foi publicado no periódico *Environmental Science and Pollution Research* em 7 de agosto de 2024 (doi.org/10.1007/s11356-024-34707-7, Fator de impacto: 5.8 e Qualis: A2), e o capítulo II encontra-se na fase de refinamento da escrita, e deve ser submetido ao *Journal of Environmental Management* (Fator de impacto: 8.7 e Qualis: A1).

Capítulo I: Rejeitos de minério de Lítio prejudicam o desenvolvimento vegetativo, a atividade fotossintética e a nutrição de espécies arbóreas.

Este estudo teve como objetivo analisar os efeitos do RML em quatro espécies lenhosas com diferentes faixas de distribuição geográfica. Além disso, avaliamos o potencial dessas espécies no contexto da fitorremediação. Para atingir esse objetivo, examinamos a atividade fotossintética, o crescimento vegetativo, a nutrição vegetal, a produção de biomassa e a tolerância no desenvolvimento inicial de duas espécies com ampla distribuição geográfica (*Enterolobium contortisiliquum* e *Handroanthus* *impetiginosus*) e duas com distribuição mais restrita (*Hymenaea courbaril* e *H. stigonocarpa*). Esperamos que espécies com ampla distribuição geográfica cultivadas em RML apresentem maior capacidade de ajuste morfofisiológico e alta tolerância devido à sua capacidade de ajuste aos diferentes ambientes em que ocorrem. Essas espécies tolerantes ao RML demonstrariam a capacidade de manter a fotossíntese, o crescimento e a produção de biomassa semelhantes às plantas cultivadas em solo não contaminado. Além disso, esperamos que essas espécies possam acumular altas concentrações de metais, mantendo sua capacidade fotossintética inalterada. Por outro lado, esperamos respostas negativas na fotossíntese, no crescimento vegetativo e na produção de biomassa em espécies com distribuição geográfica restrita, sendo consideradas menos tolerantes devido ao efeito RML.

Capítulo II: A interação intraespecífica e interespecífica facilita o crescimento inicial e absorção de metais por *Enterolobium contortisiliquum* (Vell.) Morong em rejeito de minério de lítio?

Nesse estudo, avaliamos se as interações intraespecíficas e interespecíficas podem melhorar o crescimento de *E. contortisiliquum* e ampliar seu potencial para revegetar ambientes contaminados pelo RML. Para isso, foram testados três níveis de interação: (1) *nível individual*, com um único indivíduo de *E. contortisiliquum*; (2) *nível intraespecífico*, com oito indivíduos de *E. contortisiliquum*; e (3) *nível interespecífico*, com oito indivíduos no total, sendo dois de cada uma das seguintes espécies: *E. contortisiliquum*, *Hymenaea stigonocarpa*, *Handroanthus impetiginosus* e *Hymenaea courbaril*. Esperamos que *E. contortisiliquum* apresente melhor desempenho sob cultivo interespecífico em comparação ao cultivo individual ou intraespecífico, com: (1) maior produção de biomassa, devido à melhoria das condições edáficas proporcionadas pelas espécies associadas; (2) maior eficiência fotossintética, refletida em parâmetros como fluorescência da clorofila *a* e conteúdo de clorofila; e (3) maior acúmulo de metais nos tecidos, indicando aumento do potencial fitoextrator.

Referências

Ahirwal, J., Pandey, V.C., 2021. Restoration of mine degraded land for sustainable environmental development. Restoration Ecology 29, e13268. https://doi.org/10.1111/rec.13268

Andrade, G.F., Paniz, F.P., Martins, A.C., Rocha, B.A., Da Silva Lobato, A.K., Rodrigues, J.L., Cardoso-Gustavson, P., Masuda, H.P., Batista, B.L., 2018. Agricultural use of Samarco's spilled mud assessed by rice cultivation: A promising residue use? Chemosphere 193, 892–902. https://doi.org/10.1016/j.chemosphere.2017.11.099

Anjum, S.A., Ashraf, U., Khan, I., Tanveer, M., Ali, M., Hussain, I., Wang, L.C., 2016. Chromium and Aluminum Phytotoxicity in Maize: Morpho-Physiological Responses and Metal Uptake. CLEAN Soil Air Water 44, 1075–1084. https://doi.org/10.1002/clen.201500532

Aral, H., Vecchio-Sadus, A., 2011. Lithium: Environmental Pollution and Health Effects, in: Encyclopedia of Environmental Health. Elsevier, pp. 116–125. https://doi.org/10.1016/B978-0-444-63951-6.00531-3

Ashraf, Sana, Ali, Q., Zahir, Z.A., Ashraf, Sobia, Asghar, H.N., 2019. Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils. Ecotoxicology and Environmental Safety 174, 714–727. https://doi.org/10.1016/j.ecoenv.2019.02.068

Behr, J.H., Zörb, C., 2023. Interactive effect of lithium on concentration of alkali cations in sugar beet (*Beta vulgaris L.*) under saline conditions. J. Plant Nutr. Soil Sci. 186, 38–49. https://doi.org/10.1002/jpln.202200079

Braga, P. F. A.; França S. C. A. 2013. Series studies and documents. Lithium: a strategic mineral, 81st ed, IV. CETEM/MCTI, Rio de Janeiro. (in Portuguese)

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B., Michalet, R., 2008. Facilitation in plant communities: the past, the present, and the future. Journal of Ecology 96, 18–34. https://doi.org/10.1111/j.1365-2745.2007.01295.x

Castro, E.B., Santos, L.D.T., Fernandes, L.A., Tajima, C.Y., 2016. Use of Aluminum Silicate Partial Substitution in the Substrate for Seedlings Production Corymbia citriodora. Floresta Ambient. 23, 229–236. https://doi.org/10.1590/2179-8087.106814 (in Portuguese).

Elektorowicz, M., Keropian, Z., 2015. Lithium, Vanadium and Chromium Uptake Ability of *Brassica juncea* from Lithium Mine Tailings. International Journal of Phytoremediation 17, 521–528. https://doi.org/10.1080/15226514.2013.876966

Esteves, G.D.F., De Souza, K.R.D., Bressanin, L.A., Andrade, P.C.C., Veroneze Júnior, V., Dos Reis, P.E., Da Silva, A.B., Mantovani, J.R., Magalhães, P.C., Pasqual, M., De Souza, T.C., 2020. Vermicompost improves maize, millet and *sorghum* growth in iron mine tailings. Journal of Environmental Management 264, 110468. https://doi.org/10.1016/j.jenvman.2020.110468

Ettler, V., Konečný, L., Kovářová, L., Mihaljevič, M., Šebek, O., Kříbek, B., Majer, V., Veselovský, F., Penížek, V., Vaněk, A., Nyambe, I., 2014. Surprisingly contrasting metal distribution and fractionation patterns in copper smelter-affected tropical soils in forested and grassland areas (Mufulira, Zambian Copperbelt). Science of The Total Environment 473–474, 117–124. https://doi.org/10.1016/j.scitotenv.2013.11.146

Faria, R.M., 2017. ALUMINUM SILICATE AS AN ALTERNATIVE MANAGEMENT OF WEEDS. Dissertation, Federal University of Minas Gerais. http://hdl.handle.net/1843/NCAP-ANZFX6 (in Portuguese).

Gagnon, V., Rodrigue-Morin, M., Migneault, M., Tardif, A., Garneau, L., Lalonde, S., Shipley, B., Greer, C.W., Bellenger, J.-P., Roy, S., 2020. Survival, growth and element translocation by 4 plant species growing on acidogenic gold mine tailings in Québec. Ecological Engineering 151, 105855. https://doi.org/10.1016/j.ecoleng.2020.105855

Gastauer, M., Souza Filho, P.W.M., Ramos, S.J., Caldeira, C.F., Silva, J.R., Siqueira, J.O., Furtini Neto, A.E., 2019. Mine land rehabilitation in Brazil: Goals and techniques in the context of legal requirements. Ambio 48, 74–88. https://doi.org/10.1007/s13280-018-1053-8

Ginocchio, R., León-Lobos, P., Arellano, E.C., Anic, V., Ovalle, J.F., Baker, A.J.M., 2017. Soil physicochemical factors as environmental filters for spontaneous plant colonization of abandoned tailing dumps. Environ Sci Pollut Res 24, 13484–13496. https://doi.org/10.1007/s11356-017-8894-8

Gómez, L., Contreras, A., Bolonio, D., Quintana, J., Oñate-Sánchez, L., Merino, I., 2019. Phytoremediation with trees, in: Advances in Botanical Research. Elsevier, pp. 281–321. https://doi.org/10.1016/bs.abr.2018.11.010

Gómez-Aparicio, L. 2009. The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. Journal of Ecology, 97.6, 1202-1214. https://doi.org/10.1111/j.1365-2745.2009.01573.x

Hao, H., Liu, Z., Zhao, F., Geng, Y., Sarkis, J., 2017. Material flow analysis of lithium in China. Resources Policy 51, 100–106. https://doi.org/10.1016/j.resourpol.2016.12.005

Haridasan, M., Paviani, T.I., Schiavini, I., 1986. Localization of aluminium in the leaves of some aluminium-accumulating species. Plant Soil 94, 435–437. https://doi.org/10.1007/BF02374336

Hawrylak-Nowak, B., Kalinowska, M., Szymańska, M., 2012. A Study on Selected Physiological Parameters of Plants Grown Under Lithium Supplementation. Biol Trace Elem Res 149, 425–430. https://doi.org/10.1007/s12011-012-9435-4

Heckenroth, A., Rabier, J., Dutoit, T., Torre, F., Prudent, P., Laffont-Schwob, I., 2016. Selection of native plants with phytoremediation potential for highly contaminated Mediterranean soil restoration: Tools for a non-destructive and integrative approach. Journal of Environmental Management 183, 850–863. https://doi.org/10.1016/j.jenvman.2016.09.029

Lemougna, P.N., Yliniemi, J., Ismailov, A., Levanen, E., Tanskanen, P., Kinnunen, P., Roning, J., Illikainen, M., 2019. Recycling lithium mine tailings in the production of low temperature (700–900 °C) ceramics: Effect of ladle slag and sodium compounds on the processing and final properties. Construction and Building Materials 221, 332–344. https://doi.org/10.1016/j.conbuildmat.2019.06.078

Lier, Q. J. Van. 2010. Soil Physics. 1 ed. Viçosa: Brazilian Society of Soil Science, 298p. (in Portuguese)

Lu, Y., Ranjitkar, S., Harrison, R.D., Xu, J., Ou, X., Ma, X., He, J., 2017. Selection of Native Tree Species for Subtropical Forest Restoration in Southwest China. PLoS ONE 12, e0170418. https://doi.org/10.1371/journal.pone.0170418

Martin, G., Rentsch, L., Höck, M., Bertau, M., 2017. Lithium market research – global supply, future demand and price development. Energy Storage Materials 6, 171–179. https://doi.org/10.1016/j.ensm.2016.11.004

Melo, N.M.J., Souza, J.P., 2025. Lithium Ore Tailings May Increase the Invasion Potential of *Melinis minutiflora* in Native Areas Around to Mining Sites. Austral Ecology 50, e70034. https://doi.org/10.1111/aec.70034

Meshram, P., Pandey, B.D., Mankhand, T.R., 2014. Extraction of lithium from primary and secondary sources by pre-treatment, leaching and separation: A comprehensive review. Hydrometallurgy 150, 192–208. https://doi.org/10.1016/j.hydromet.2014.10.012

Meyer, S.T., Castro, S.R., Fernandes, M.M., Soares, A.C., De Souza Freitas, G.A., Ribeiro, E., 2016. Heavy-metal-contaminated industrial soil: Uptake assessment in native plant species from Brazilian Cerrado. International Journal of Phytoremediation 18, 832–838. https://doi.org/10.1080/15226514.2016.1146224

Mukhopadhyay, S., Masto, R.E., Yadav, A., George, J., Ram, L.C., Shukla, S.P., 2016. Soil quality index for evaluation of reclaimed coal mine spoil. Science of The Total Environment 542, 540–550. https://doi.org/10.1016/j.scitotenv.2015.10.035

Naranjo, M.A., Romero, C., Bellés, J.M., Montesinos, C., Vicente, O., Serrano, R., 2003. Lithium treatment induces a hypersensitive-like response in tobacco. Planta 217, 417–424. https://doi.org/10.1007/s00425-003-1017-4

Nardi, S., Pizzeghello, D., Muscolo, A., Vianello, A., 2002. Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry 34, 1527–1536. https://doi.org/10.1016/S0038-0717(02)00174-8

Navarro-Cano, J.A., Goberna, M., Verdú, M., 2019. Using plant functional distances to select species for restoration of mining sites. Journal of Applied Ecology 56, 2353–2362. https://doi.org/10.1111/1365-2664.13453

Navarro-Cano, J.A., Verdú, M., Goberna, M., 2018. Trait-based selection of nurse plants to restore ecosystem functions in mine tailings. Journal of Applied Ecology 55, 1195–1206. https://doi.org/10.1111/1365-2664.13094

Nunes, F.C., Alves, L.D.J., Prasad, M.N.V., De Carvalho, C.C.N., Nolasco, E.C., 2022. Phytoremediation Strategies for Rehabilitation of Soils Affected by Red Mud: the Mariana Tailing Dam Collapse (Minas Gerais, Brazil). Eurasian Soil Sc. 55, 673–685. https://doi.org/10.1134/S1064229322050052

Pourret, O., Lange, B., Bonhoure, J., Colinet, G., Decrée, S., Mahy, G., Séleck, M., Shutcha, M., Faucon, M.-P., 2016. Assessment of soil metal distribution and environmental impact of mining in Katanga (Democratic Republic of Congo). Applied Geochemistry 64, 43–55. https://doi.org/10.1016/j.apgeochem.2015.07.012

Raimondi, I. M. 2014. Geological and geotechnical study and characterization in tailing: Adrianópolis (PR). Master's Dissertation, São Carlos School of Engineering, University of São Paulo, São Carlos.

doi:10.11606/D.18.2014.tde-17092014-093634. Retrieved 2023-05-06, from www.teses.usp.br (in Portuguese).

Rosado, L.S.S., Modolo, G.S., Guimarães, Z.T.M., Dos Santos, V.A.H.F., Rosado, S.I.P., Ferreira, M.J., 2023. Short-term responses of native tree species to site conditions after mining in the Central Amazon. Ecological Engineering 192, 106992. https://doi.org/10.1016/j.ecoleng.2023.106992

Roy, R. N., Saha, B. 2019. Plants response to heavy metal stress, in: Stress physiology of woody plants. CRC Press, pp. 203-258. https://doi:10.1201/9780429190476-10

Roy, T., Plante, B., Benzaazoua, M., Demers, I., 2023. Geochemistry and mineralogy of a spodumene-pegmatite lithium ore at various mineral beneficiation stages. Minerals Engineering 202, 108312. https://doi.org/10.1016/j.mineng.2023.108312

Salt, D.E., Smith, R.D., Raskin, I., 1998. PHYTOREMEDIATION 26.

Siljkovic, B., Denic, N., Rakic, G., 2017. Environmental and economic assessments the effect of critical mineral of green revolution: Lithium. Min Metal Eng Bor 103–114. https://doi.org/10.5937/mmeb1702103S

Tanveer, M., Hasanuzzaman, M., Wang, L., 2019. Lithium in Environment and Potential Targets to Reduce Lithium Toxicity in Plants. J Plant Growth Regul 38, 1574–1586. https://doi.org/10.1007/s00344-019-09957-2

Yan, A., Wang, Y., Tan, S.N., Mohd Yusof, M.L., Ghosh, S., Chen, Z., 2020. Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. Front. Plant Sci. 11, 359. https://doi.org/10.3389/fpls.2020.00359

Young, R.E., Gann, G.D., Walder, B., Liu, J., Cui, W., Newton, V., Nelson, C.R., Tashe, N., Jasper, D., Silveira, F.A.O., Carrick, P.J., Hägglund, T., Carlsén, S., Dixon, K., 2022. International principles and standards for the ecological restoration and recovery of mine sites. Restoration Ecology 30, e13771. https://doi.org/10.1111/rec.13771

Zanchi, C.S., Silva, A.O., Batista, É.R., Peixoto, D.S., Barbosa, M.V., Dos Santos, J.V., Alvarenga, I.F.S., Silva, B.M., Carneiro, M.A.C., 2022. Pre-cultivation with Herbaceous Plants Assists in the Revegetation Process of Iron Mining Tailings with *Enterolobium contortisiliquum*. Water Air Soil Pollut 233, 231. https://doi.org/10.1007/s11270-022-05696-5

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CAPÍTULO I

Lithium ore tailings harm the vegetative development, photosynthetic activity, and nutrition of tree species

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LITHIUM ORE TAILINGS HARM THE VEGETATIVE DEVELOPMENT, PHOTOSYNTHETIC ACTIVITY, AND NUTRITION OF TREE SPECIES

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Abstract

Lithium (Li) exploitation promotes socioeconomic advances but may result in harmful environmental impacts. Thus, species selection for recovering environments degraded by Li mining is essential. We investigated the tolerance and early growth of four tree species to Li ore tailings (LOT), *Enterolobium contortisiliquum* and *Handroanthus impetiginosus* with wide geographic distribution and *Hymenaea courbaril* and *H. stigonocarpa* with restricted geographic distribution. The plants grew in LOT and soil for 255 days to evaluate photosynthesis, growth, and mineral nutrition. LOT negatively affected species growth, reducing the length of stems, roots, and biomass through structural and nutritional impoverishment. LOT favored the accumulation of Mg and decreased the absorption of K. The species presented a reduction in potential quantum efficiency and the chlorophyll index (b and total). *E. contortisiliquum* was the least tolerant species to LOT, and *H. courbaril* and *H. stigonocarpa* maintained their mass production in LOT, indicating greater tolerance to tailing. Furthermore, *H. courbaril* presented a translocation factor > 1 for Li and Mn, indicating the potential for phytoextraction of these metals. Our results offer first-time insights into the impacts of LOT on the early development of tree species with different geographic distribution ranges. This study may help the tree species selection with a phytoremediation role, aiming to recover areas affected by Li's mining activity.

Keywords: Biomass, Chlorophyll, Functional traits, Lithium ore mining, Metals, Nutrient absorption, Photosynthesis, Tolerance indices.

Introduction

Lithium (Li) is a metal widely used worldwide, and its global exploitation has increased in recent years, mainly due to its application in technological items such as batteries (Zhao et al., 2017; Martin et al., 2017). The Li extraction and subsequent processing into Li carbonate and hydroxide, generate tailings and chemical residues (Braga and França, 2013). The resulting ore tailings are named aluminum silicate, a subproduct of Li extraction (Castro et al., 2016). Although it contains some essential nutrients such as phosphorus, potassium, calcium, magnesium, and other metals such as manganese and sodium in its chemical composition, the waste still retains approximately 1% of Li after the extraction processes (Castro et al., 2016).

Although different nutrients are essential for plant growth, their toxicity depends on their role in plant development and may vary according to their ambient concentration (Roy et al., 2019). Metals could affect morphophysiological traits positively or negatively, and this response is plant-dependent (Anjum et al., 2016; Esteves et al., 2020; Rosado et al., 2023). Tree species with narrow geographical distribution may present leaf necrosis and reduced dry mass production in soils contaminated with high cadmium and zinc concentrations. On the other hand, species with wide geographical distribution may maintain biomass production in contaminated soils, accumulating the metals in their roots (Meyer et al., 2016). *Enterolobium contortisiliquum* (Vell.) Morong, a widely distributed tree in Brazil, showed the ability to tolerate and efficiently absorb sodium, copper, and zinc, being recently indicated for revegetation of tailings areas (Silva et al., 2018; Zanchi et al., 2022). Species with a wide geographical distribution are possibly more tolerant to metals in soil due to the broad range of environmental conditions in which they occur (Scalon et al., 2005; Brack and Grings 2011; Zanchi et al., 2022).

Li is not considered an essential element for plants, and its effects on tree species are poorly understood, with most studies focusing only on herbaceous plants or cultivars (Shahzad et al., 2016; Tanveer et al., 2019; Kastori et al., 2022; Shakoor et al., 2023a). At high concentrations, Li reduced root growth and development in *Helianthus annuus* L. (Hawrylak-Nowak et al., 2012), resulting in curling and chlorosis in old leaves of *Nicotiana tabacum* L. (Naranjo et al., 2003), and reduced content of chlorophyll a and b in *Brassica carinata* A. Braun (Li et al. 2009). However, low Li concentrations stimulated root growth and increased plant biomass in *Helianthus annuus* L. (Hawrylak-Nowak et al., 2012; Tanveer et al., 2019).

With increasing environmental impacts in response to mining processes, the recovery of impacted areas is necessary to mitigate the losses on biodiversity (Ilunga et al., 2015). The recovery of impacted environments by mining may be a challenge due to the rigorous conditions imposed by the degraded environment, such as increased concentration of pollutants, high solar radiation, low aggregation or soil compaction, and low vegetation cover (Ettler et al., 2014; Mukhopadhyay et al., 2016; Pourret et al., 2016). These modifications limit the establishment of less tolerant species or species without specific adaptations (Ginocchio et al., 2017; Heckenroth et al., 2016; Lu et al., 2017; Padilla et al., 2009). Several studies have been carried out to mitigate these impacts (Funk et al., 2008; Montoya et al., 2012; Laughlin, 2014; Lu et al., 2017). However, traditional approaches based on native species reintroduction without considering functional traits do not guarantee the recovery of the original ecosystem (Carlucci et al., 2020).

Parameters related to growth, biomass production, plant nutrition, and photosynthetic activity have made it possible to investigate the tolerance of species to environments contaminated by metals from mining activities (Meyer et al., 2016; Esteves et al., 2020; Rosado et al., 2023). The investigation and selection of tolerant species can contribute to phytoremediation studies in environments affected by mining. Phytoremediation consists of selecting plants that have the potential for decontamination by removing, immobilizing, or making contaminants harmless to the ecosystem (Salt et al., 1998). In the long term, woody species are a better alternative for phytoremediation, as they generally retain the contaminant in the root and stem, and it is not necessary to carry out management plans or later collect the material (Gómez et al., 2019). Plant species that manage to develop and establish themselves in environments contaminated by metals must have resistant mechanisms that allow them to circumvent phytotoxicity (Bovet et al., 2006), such as tolerance, metal accumulation, and maintenance of photosynthetic activity or morphological plasticity (Haridasan et al.,1986; Andrade et al., 2018).

In Brazil, there are large reserves of Li located mainly in the north of Minas Gerais state (Braga and França, 2013). Even though Li mining favors socioeconomic advances such as increased Human Development Index in the region where Li is mined, this activity promotes negative socioenvironmental impacts. Mining activity promotes deforestation, the removal of native species, and the production of waste containing high concentrations of chemical elements. The tailings deposit can form increasing sources of environmental contamination, mainly affecting the soil and associated vegetation (Aral and Vecchio-

Sadus, 2008). In general, there is a lack of studies on the environmental disturbance of Li mining, especially about chemical contamination (Agusdinata et al., 2018). Most existing research on Li mining in Brazil addresses its history, material processing, economic demand, and use in civil construction (Braga and França 2013; Martin et al., 2017; Lemougna et al., 2019).

This study aimed to analyze the effects of Li ore tailings (LOT) on four woody species with different geographic distribution ranges. Furthermore, we intend to evaluate the potential of these species in the context of phytoremediation. To achieve this objective, we examined photosynthetic activity, vegetative growth, plant nutrition, biomass production, and tolerance in the early development of two species with a wide geographic distribution and two with a more restricted distribution. We expect that wide geographic distribution species grown in LOT present greater adjustment capacity in morphophysiological traits and high tolerance in response to the wide range of environments in which they occur. These tolerant species to LOT would demonstrate the ability to maintain photosynthesis, growth, and biomass production similar to plants grown in uncontaminated soil. Also, we expect these species may accumulate high metal concentrations, keeping their photosynthetic capacity unchanged. On the other hand, we expect negative responses in leaf photosynthesis, vegetative growth, and biomass production in species with a narrow geographical distribution which is considered less tolerant due to the LOT effect.

Material and methods

Growth conditions and plant material

The experiment was carried out at the Plant Functional Ecology Laboratory (LEFuP) at the Federal University of Viçosa, campus Florestal (UFV/CAF, 19°52'29" S, and 44°25'12" W), in open chambers (OC). The climate in the area is classified as subtropical, with dry winters and rainy summers.

We used two substrates in the experiment: a treatment (lithium ore tailings – LOT) and a control (reference soil - RS). The LOT used as treatment was provided by the *Companhia Brasileira de Litio* (CBL) in March 2022. The reference soil used for the control was taken from an area within the Federal University of Viçosa, *campus* Florestal. Substrate analyses (Table 1) were performed by the *Soloquímica – analysis for*

Agriculture and the Environment laboratory (Brasília, Brazil) according to EMBRAPA SOLOS methodologies (Silva et al., 2009; Donagema et al., 2011).

Table 1: Fertility, organic matter, and granulometry of substrates (LOT and RS) used for the experiment.

Characteristics	**SUBSTRATES**	
	Lithium ore tailings (LOT)	**Reference Soil (RS)**
pH (H_2O)	7.700	5.100
V (%)	99.000	35.333
m (%)	0.000	12.000
SB ($cmol_c\ dm^{-3}$)	130.433	2.233
T ($cmol_c\ dm^{-3}$)	131.933	6.267
H+Al ($cmol_c\ dm^{-3}$)	1.500	4.000
Al^{+3} ($cmol_c\ dm^{-3}$)	0.000	0.267
Ca ($cmol_c\ dm^{-3}$)	128.300	1.800
Mg ($cmol_c\ dm^{-3}$)	1.100	0.300
K ($cmol_c\ dm^{-3}$)	0.257	0.100
Na ($cmol_c\ dm^{-3}$)	0.760	0.050
B ($mg\ dm^{-3}$)	0.460	0.193
Cu ($mg\ dm^{-3}$)	0.267	0.500
Fe ($mg\ dm^{-3}$)	1.767	81.667
Mn ($mg\ dm^{-3}$)	67.633	9.000
Zn ($mg\ dm^{-3}$)	1.400	0.867
S ($mg\ dm^{-3}$)	484.267	58.967
P-rem ($mg\ dm^{-3}$)	9.097	0.798
P ($mg\ dm^{-3}$)	1.800	1.267
N ($mg\ dm^{-3}$)	583.333	630.000
Li ($mg\ dm^{-3}$)	262.377	4.226
C ($g\ kg^{-1}$)	3.733	5.633
OM ($g\ kg^{-1}$)	6.400	9.700
Granulometry		
Clay ($g\ kg^{-1}$)	125.000	450.000
Coarse sand ($g\ kg^{-1}$)	130.667	94.667
Thin sand ($g\ kg^{-1}$)	125.333	64.000
Silt ($g\ kg^{-1}$)	619.000	391.333

pH: hydrogenion potential in H_2O; V: base saturation; m: aluminum saturation; SB: sum of bases (Mg, Ca, K, Na), T: total cation exchange capacity; H + Al: potential acidity; Al^{3+}: aluminum; Ca: calcium; Mg: magnesium; K: potassium; Na: sodium; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; S: sulfur; P-rem: remaining phosphorus; P: phosphorus; OM: organic matter; C: carbon; N: nitrogen, and Li: lithium.

The species selection for the experiment was based on field expeditions in the municipality of Divisa Alegre (15°43'34" S, and 41°20'19" W, the main area affected by the tailings deposit from Li mining activities), in the predominant vegetation of this area (Atlantic Forest and Cerrado), and field reports from the CBL. Four species were selected, two with a wide geographical distribution (*Enterolobium contortisiliquum* (Vell.) Morong and *Handroanthus impetiginosus* (Mart. ex DC.) Mattos) and two with a more restricted geographical distribution (*Hymenaea stigonocarpa* Mart. ex Hayne and *Hymenaea courbaril* L., (Table S1). All seeds of the selected species were purchased from the Arbocenter company in May 2022 (seeds collected in 2020).

The seeds of *E. contortisiliquum*, *H. stigonocarpa*, and *H. coubaril* undergo pre-germination treatment to break physical dormancy on May 10, 2022 (Silva et al., 2012; Moreira e Oliveira, 2016; Motta et al., 2019). The seeds of these species were mechanically scarified on the opposite side of the hilum with sandpaper to avoid damage to the hypocotyl-radicle axis. Seeds of all four species were sown directly in pots with a capacity for 0,01 m^3 of the substrate in both treatments. Ninety-six pots were used, 12 pots in each substrate (12 with LOT and 12 with reference soil) for each species. The pots were distributed in eight OCs, four OCs with LOT pots, and four OCs with RS pots. In each OC, three pots of each species were distributed, totaling 12 pots. The pots with three seeds were irrigated daily during the experimental period. To minimize the influence of the position of the pots within each OC, pot rotation between OCs was performed every two months.

After the emergence period, when the seedlings were approximately two months old, only one plant was kept per pot. Our sample number consisted of 24 plants per species, 12 in each substrate (LOT *vs* RS).

Chlorophyll a fluorescence and chlorophyll indices

Measurements of chlorophyll *a* fluorescence and chlorophyll content index were determined at 135 DBE and 255 DBE. In the first data collection (135 DBE), 64 plants were used, 32 in each substrate (n=8 plants per species in each substrate). In the second data collection (255 DBE), 80 plants were measured, 40 in each substrate (n=10 plants per species in each substrate).

The chlorophyll *a* fluorescence was measured with a portable fluorometer model PARFluorPen FP110 (Photon System Instruments, Czech Republic), following the NPQ1 protocol. One leaf or leaflet per plant on each substrate was acclimated to darkness with metal clips placed in the central region of fully expanded leaflets for 30 min. After leaf acclimation, the minimum fluorescence level (F0) was measured during a dark period. Afterward, a short saturating flash of light (intensity 50% = 1.500 µmol m^{-2} s^{-1}) was applied to reduce the plastoquinone pool and measure maximum fluorescence in the dark-adapted state (F_m). The values obtained were used to determine maximum quantum yield of PSII - F_v/F_m (($F_m - F_0)/F_m$, Oxborough and Baker, 1997). After a short dark relaxation, the leaf was exposed to actinic irradiance (intensity 10% = 100 µmol m^{-2} s^{-1}) for 60s to stimulate a transient state called the Kautsky effect. Then, during exposure to actinic light, a sequence of 5 pulses of saturating flashes (intensity 50% = 1.500 µmol m^{-2} s^{-1}) was applied at 12s intervals to probe NPQ and ϕPSII in a light-adapted state. The electron transport rate (ETR) was calculated using the following formula: ETR= ϕPSII x PAR x 0.5 x la; where 0.5 is the proportion of photons destined for the two photosystems (Melis et al., 1987), la is the leaf absorbance, and PAR is the photosynthetically active radiation.

The chlorophyll content index was determined using an electronic chlorophyll content meter (clorofiLOG, model CFL1030; FALKER, Porto Alegre, BR). Chlorophyll *a*, *b*, and *total* were measured in the same plants and leaves used for chlorophyll *a* fluorescence measurement. Chlorophyll indices were determined optically via detectable light frequency for chlorophylls. All measurements were performed between 7:00 and 11:00 am.

Vegetative morphometry

The leaf number (LN), stem diameter (SD), and stem length (SL) were measured weekly over eight months (May 25, 2022, to January 25, 2023, a total of 255 days after the beginning of the experiment, DBE) in 12 plants per species in both substrates (LOT and RS). The length and width of the eophylls and two leaves (metaphylls) were monitored weekly from emergence to full growth (leaves with growth stabilized). The leaf stabilization period was defined as the leaf expansion interval (LEI, days $^{-1}$). Then, the leaf area was determined after the leaf showed growth stabilization (LA=stabilized width multiplied by the stabilized length). The leaf expansion rate was calculated as the product of LA/LEI (LER=LA/LEI, cm^2 days $^{-1}$). The time from the leaf emergence in the branch until its fall was defined as the leaf life span (LLS, days).

Nutrient concentration and translocation factor

We selected the nutrients and metals with the highest concentrations in the LOT to determine their concentration in the plant tissue (root, stem, and leaf) of each species in both substrates. We separated triplicates of root, stem, and leaf samples. The samples were dried in an oven for 72 hours at 60 °C until constant weight (He et al., 2015). Subsequently, they were ground in a Wiley mill, sieved with a 1.0 mm mesh (20 mesh), and stored in glass vials for chemical analysis (Carmo et al., 2000). In our study, we determined the concentrations of Calcium [Ca]; Potassium [K], Magnesium [Mg], Phosphorus [P], Nitrogen [N], Aluminum [Al], Manganese [Mn], and Lithium [Li].

The samples were subjected to wet digestion with HNO_3 and $HClO_4$ (Nitro-Perchloric Digestion, Carmo et al., 2000). From the digested material, it was possible to determine the elements Ca, K, Mg, P, Al, Mn, and Li dissolved in the acid solution and determined in the Agilent MP-AES 4200 Spectrometer (Liberato et al., 2017). The Agilent MP-AES is a compact microwave-induced plasma atomic emission spectrometer based on a highly sensitive nitrogen plasma and an alternative to Flame Atomic Absorption Spectrometry (FAAS), allowing multielementary analysis (Liberato et al., 2017). Nitrogen was determined by the Kjeldahl method and titration after digestion with $H_2 SO_4 + H_2O_2$ (Kjeldahl - Sulfuric Digestion, Carmo et al., 2000). All chemical analyses of the plant tissue were carried out at the *Soloquímica – analysis for Agriculture and the Environment* laboratory (Brasília, Brazil).

The Translocation Factor (TF) was calculated at the end of the experiment (255 DBE), considering the concentration of nutrients (Ca, K, Mg, P, and N) and metals (Al, Mn, and Li) in the root, stem, and leaf of each species on both substrates (LOT and RS). TF was used to determine the mobilization or translocation of nutrients and metals from the roots to the aerial part of the plants (Gupta et al., 2008). The TF calculation was based on the equation used by Meyer et al., (2016): TF = Nutrient or metal concentration in the aerial part (leaf + stem) (mg/kg) / Nutrient or metal concentration in the root (mg/kg). The values > 1 demonstrate that the plant is translocating nutrients or metals to the aerial part (Elektorowicz and Keropian, 2015).

Leaf area, biomass, and biometric indices

The leaf area of each species was measured at the end of the experiment (255 DBE) in 12 plants of each species in each substrate (LOT and RS), except for *H. stigonocarpa* (11 plants in each substrate) and *H. impetiginosus* (11 plants in the LOT and 12 plants in the RS), since both had different numbers of plants at the end of the experiment. The leaf area was measured after their petioles were removed. Subsequently, the leaf blade was scanned in black and white on a scanner. Then, the total leaf area (TLA) and average leaf area (ALA) were calculated using ImageJ 1.x software (Schneider; Rasband and Eliceiri, 2012). The same plants used to measure the leaf area were separated into stem and root to determine the length of the stem (SL, cm) and root (RL, cm), stem dry mass (SDM, g), leaf dry mass (LDM, g), root dry mass (RDM, g), and total dry mass (TDM, g). The leaves, stems, and roots were dried in an oven for 72 hours at 60 °C until constant weight (He et al., 2015). The masses were determined on a semi-analytical balance at the LEFuP/UFV. The TDM was determined by the sum of the dry mass of the stem, leaf, and root of each plant. After all the measurements, the biometric indices were calculated: leaf area ratio (LAR, total leaf area/total dry mass, cm^2 g^{-1}), specific leaf area (SLA, leaf area/leaf dry mass, cm^2 g^{-1}), and root/shoot ratio (RSR, root dry mass/shoot dry mass).

Tolerance Index

The tolerance indices (TI) were calculated at the end of the experiment (255 DBE), considering the dry mass of the plants grown in the two substrates. TI was calculated in the same plants used for biomass measurements. Based on the dry mass weight, tolerance indices were calculated for the stem (STI), root (RTI), leaf (LTI), and total dry mass (TTI). The tolerance indices were based on the equation used by Meyer et al., (2016), proposed by Nautiyal et al., (2002): TI= dry mass (plant organ) in the tailings/dry mass (plant organ) in the soil.

Statistical analyzes

The mean and standard deviation were calculated for physiological measurements, vegetative morphometry, leaf growth, biomass, biometric indices, tolerance indices, the concentration of nutrients and metals in plant tissue, and translocation factor. Subsequently, the Shapiro-Wilk test was applied to test the normal distribution of the data set. When the data set did not show normality, we transformed the data set into logarithm base 10. Afterward, Levene's test was applied to verify the homogeneity of variance. For vegetative morphometry and physiological data set, a mixed two-way ANOVA with repeated measures over time was used (considering species and substrate between-subject factors and time as the within-subject factor). A posteriori, Tukey's test was performed to investigate significant differences ($p<0.05$) between species and substrates over time (DBE). We used the three-way or two-way ANOVA (species, substrate, and plant tissue as factors) to seek differences in the nutrients or metals concentration in plant tissue, with a Tukey's test to compare significant differences ($p<0.05$) between treatments. For leaf growth, biomass (root, stem, and leaf), biometric indices, and translocation factor, a two-way ANOVA (using species and substrate as factors) and Tukey's test were used to compare significant differences ($p<0.05$) between species and substrate. For the tolerance indices, a one-way ANOVA was performed with a posteriori Tukey's test with a significance level of 5% to compare differences between species. The R software version R - 4.2.2 – win (R Development Core Team, 2020) was used to perform all statistical analyses. Graphs were created using SigmaPlot software version 10 (Systat Software, San Jose, CA). The graphs were built up according to the result of the statistical analysis. The summary of the statistical analyses is available in the supplementary material (Table S2).

Results

Chlorophyll a fluorescence and chlorophyll indices

Regardless of the species, the F_v/F_m was higher in RS than in LOT (Fig. S1b). *Hymenaea courbaril* and *H. stigonocarpa* did not show differences in ΦPSII and ETR between substrates. Among the species, *E. contortisiliquum* showed lower values of ΦPSII and ETR in the RS at 135 DBE, and *H. impetiginosus* at 255 DBE (Fig. S2a-d and Fig. S2e-h, interaction among species, substrate, and DBE). At 135 DBE, *E. contortisiliquum* had higher ΦPSII and ETR in LOT than in RS, but at 255 DBE, *H. impetiginosus* showed higher ΦPSII and ETR in LOT than in RS (Fig. S2a, e, and Fig. S2d, h, interaction among substrate, DBE, and species). The plants grown in LOT and RS did not change NPQ values (Fig. S3).

Enterolobium contortisiliquum showed higher chlorophyll a in the LOT (Table 2, interaction between species and substrate), but only *H. courbaril* showed lower chlorophyll a in the LOT than in the RS (Table 2, interaction between substrate and species). Regardless of species, the chlorophyll b and total were higher in the RS than in the LOT at 255 DBE (Table 2, interaction between substrate and DBE). Furthermore, there was an increase in chlorophyll b and total from 135 DBE to 255 DBE in the RS (Table 2, interaction between DBE and substrate).

Table 2: Mean and standard deviation of chlorophyll index (a, b, and total) by species in each substrate and DBE (LOT= Lithium ore Tailings and RS= Reference Soil). All values are means of n = 8 ± SD.

SUBSTRATE	**SPECIES**	**135 DBE**			**255 DBE**		
		Chlorophyll *a*	**Chlorophyll *b***	**Chlorophyll *total***	**Chlorophyll *a***	**Chlorophyll *b***	**Chlorophyll *total***
LOT	*E. contortisiliquum*	24.9 ± 11.1 **Aa**	5.6 ± 5.9 **Aa**	22.8 ± 8.5 **Aa**	48.9 ± 27.4 **Aa**	3.8 ± 2.6 **Ab**	52.7 ± 27.6 **Ab**
	H. courbaril	28.0 ± 7.2 **Bb**	5.9 ± 4.0 **Aa**	33.9 ± 10.9 **Aa**	23.0 ± 5.3 **Bb**	3.6 ± 0.8 **Ab**	26.6 ± 6.0 **Ab**
	H. stigonocarpa	27.3 ± 5.5 **ABa**	5.2 ± 1.6 **Aa**	32.6 ± 7.2 **Aa**	28.9 ± 7.0 **ABa**	4.6 ± 1.7 **Ab**	33.6 ± 8.5 **Ab**
	H. impetiginosus	28.9 ± 4.2 **Ba**	5.6 ± 1.6 **Aa**	34.5 ± 5.6 **Aa**	19.0 ± 6.5 **Ba**	2.9 ± 0.6 **Ab**	21.9 ± 6.5 **Ab**
RS	*E. contortisiliquum*	23.7 ± 7.8 **Aa**	3.66 ± 1.2 **Aa**	27.4 ± 7.5 **Aa**	42.1 ± 13.8 **Aa**	14.0 ± 8.2 **Aa***	56.1 ± 19.2 **Aa***
	H. courbaril	33.8 ± 7.2 **Aa**	9.64 ± 9.8 **Aa**	42.7 ± 14.9 **Aa**	38.2 ± 7.9 **Aa**	10.1 ± 4.6 **Aa***	48.3 ± 12.5 **Aa***
	H. stigonocarpa	32.2 ± 6.3 **Aa**	6.39 ± 2.7 **Aa**	38.6 ± 8.6 **Aa**	38.2 ± 9.0 **Aa**	11.0 ± 3.5 **Aa***	49.2 ± 11.6 **Aa***
	H. impetiginosus	24.3 ± 5.7 **Aa**	4.14 ± 1.2 **Aa**	28.4 ± 6.8 **Aa**	31.5 ± 4.8 **Aa**	5.5 ± 1.1 **Aa***	37.0 ± 5.6 **Aa***

Interactions ($p<0.05$): Chlorophyll a = species:substrate; Chlorophyll b = substrate:DBE; and Chlorophyll substrate:DBE.

Capital letters indicate differences between species in each substrate, in each response variable.

Lowercase letters indicate differences between substrates.

* Indicate differences between DBE in each substrate.

Vegetative morphometry and growth over time

Enterolobium contortisiliquum had a higher LN from 45 to 195 DBE than the other species in both substrates (except *H. impetiginosus* in soil at 165 DBE). All plants in the RS increased LN over time. In LOT, only *E. contortisiliquum* did not show an increase in LN after 75 DBE (Fig. 1a-d, interaction among species, substrate, and DBE). *Enterolobium contortisiliquum* showed higher LN when growing in LOT than in RS from 105 to 135 DBE, but from 195 DBE, the plants grown in RS showed higher LN than those in LOT (Fig. 1a, interaction among substrate, species, and DBE). *Hymenaea courbaril* and *H. impetiginosus* showed higher LN in RS than in LOT from 195 to 255 DBE; *H. stigonocarpa* showed no differences in LN between the substrates (Fig. 1b, d, and Fig. 1c, interaction among substrate, species and DBE). Leaf development differed only between species (Fig. S4).

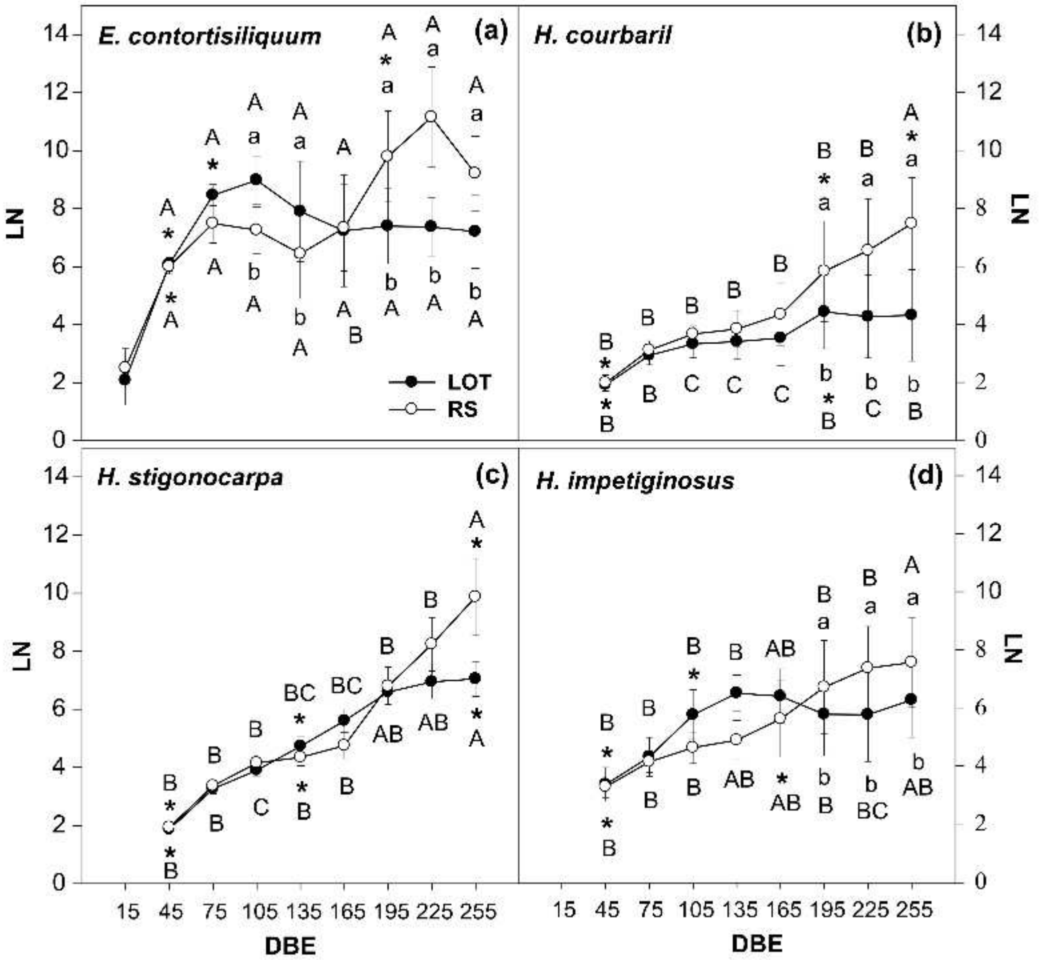

Fig. 1 Leaf production over time in four woody species grown in Lithium Ore Tailings (LOT) and reference soil (RS) in open chambers. LN = leaf number; (a) *E. contortisiliquum*; (b) *H. courbaril*; (c) *H. stigonocarpa*, and (d) *H. impetiginosus*. ● averages of the species in the LOT ○ averages of the species in the RS. The bars indicate the standard deviation. Capital letters indicate differences between species within the same DBE and on the same substrate. Lowercase letters indicate differences between substrates in the same DBE for each species. * Indicate differences in each substrate over time (DBE) by species.

Concentrations of nutrients and metals in plant tissue and translocation factor

The [Li] was higher in all tissues of the species in LOT than in RS (except in the leaf of *E. contortisiliquum* and the leaf and stem of *H. stigonocarpa*). *Enterolobium contortisiliquum* showed higher [Li] in the stem, and *H. stigonocarpa* in the root (Fig. 2a-d interaction between tissue and substrate). However, *H. impetiginosus* presented the highest [Li] (>100 mg/kg) regardless of tissue (Fig. 2c, simple effect of substrate).

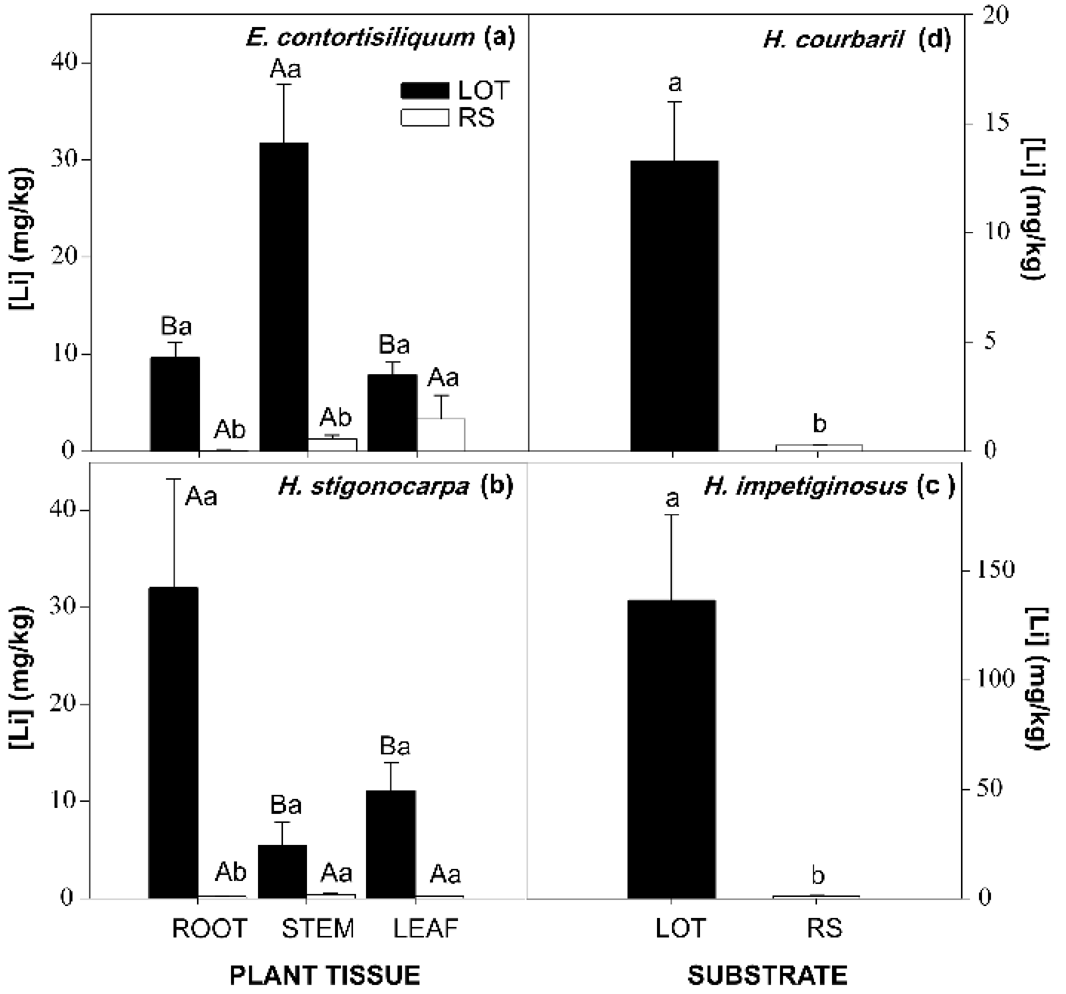

Fig. 2 Li concentration (mg/kg) in four woody species grown in LOT (Lithium Ore Tailings) and reference soil (RS) in open chambers. [Li] = lithium concentration; (a) *E. contortisiliquum*; (b) *H. courbaril*; (c) *H. stigonocarpa*, and (d) *H. impetiginosus*. The bars represent the mean and standard deviation. Means and deviations refer to triplicates of 1g of dry weight per sample. Capital letters compare differences between tissues and lowercase letters compare differences between substrates, $p<0.05$.

All species show higher [Ca], [P], and [Mg] in the LOT than in the RS in at least one of the plant tissues, except for *H. courbaril* and *H. stigonocarpa* where [Mg] does not differ between substrates in any of the tissues (Table 3, interaction among substrate, tissue, and species). The species showed greater [K], [N], [Al], or [Mn] in the RS than in the LOT. The [K] and [N] were higher in the leaves of all species (except for *E. contortisiliquum*). [Al] was higher in the root of *H. impetiginosus*, while [Mn] was higher in *H. courbaril* and *H. stigonocarpa*, regardless of tissue (Table 3, interaction among substrate, species, and tissue).

In the LOT, *E. contortisiliquum* and *H. impetiginosus* presented higher [Ca] in the leaves, with the highest [Ca] in the roots occurring in *H. impetiginosus* and in the stem of *E. contortisiliquum* in both substrates (Table 3, interaction among species, tissue, and substrate). In the LOT, the highest [P] in the leaves was found in *E. contortisiliquum* and in the stem in *E. contortisiliquum* and *H. courbaril* (Table 3, interaction among species, tissue, and substrate). *Handroanthus impetiginosus* showed higher [K] in the roots, stem, and leaf than the other species in both substrates, except for the root and leaves of *E. contortisiliquum* in the LOT and stem of *H. courbaril* in the RS (Table 3, interaction among species, tissue, and substrate). In the LOT, *E. contortisiliquum* and *H. impetiginosus* exhibited higher [N] in leaves, while in the RS, the lowest [N] in leaves occurred in *E. contortisiliquum* (Table 3, interaction among species, tissue, and substrate). In the LOT, *E. contortisiliquum* presented higher [Mg] in the leaves than the other species (Table 3, interaction among species, tissue, and substrate).

In LOT, except for *H. impetiginosus*, all species presented higher [Mn] in leaves than in roots (Table 3, interaction among tissue, substrate, and species). In LOT, [Ca] was higher in the leaves of *H. stigonocarpa* and lower in the roots of *H. courbaril* and *H. impetiginosus*. In *E. contortisiliquum*, [Ca] was lower in roots in both substrates (Table 3, interaction among tissue, substrate, and species). In LOT, *H. impetiginosus* presented higher [P] and [N] in leaves than in other tissues; *E. contortisiliquum* also showed higher [N] in the leaves, but [P] was higher in the roots. *Hymenaea courbaril* had a higher [P] only in the stem, and *H. stigonocarpa* had a higher [N] only in the root. However, in the RS, all species presented higher [P] and [N] (except *H. impetiginosus* for [P]) in leaves than in other tissues (Table 3, interaction among tissue, substrate, and species). In the RS, *H. courbaril* had the lowest [K] in the roots, while in *H. impetiginosus* the highest [K] occurred in the leaves (Table 3, interaction among tissue, substrate, and species).

All species translocated Ca, K, Mg, Al, P, N, Mn, and Li regardless of the substrate, except *H. impetiginosus* which did not translocate Al in the RS and Li in the LOT; and *H. stigonocarpa* that did not translocate N and Li in the LOT (Table 4). In the LOT, *E. contortisiliquum* translocated more Mg and N than the other species (Table 4, interaction between species and substrate). All species, except *H. courbaril*, showed a greater Mg translocation in LOT than in RS (Table 4, interaction between substrate and species). *Enterolobium contortisiliquum* had a greater N translocation in the LOT than in the RS, while *H. stigonocarpa* and *H. courbaril* translocated more N in the RS than in the LOT (Table 4, interaction between substrate and species).

Table 3. Mean and standard deviation of the concentration of nutrients and metals (mg/kg) for species in each substrate (LOT= Lithium ore tailing and RS= Reference Soil). Nutrient or metal content is given per 1g dry weight of the sample. All values are means of triplicates ± SD. The highlight in gray refers to the root in dark, stem in gray, and leaves in white.

Substrate	**Species**	**Plant tissue**	**Concentration of nutrients and metals**						
			Ca	**K**	**Mg**	**Al**	**P**	**N**	**Mn**
LOT	*E. contortisiliquum*	ROOT	1823.3 ± 616.5 **Ba***	6656.6 ± 393.2 **Aa**	314.8 ± 49.5 **Aa**	306.8 ± 137.0 **Aa**	461.6 ± 111.9 **Aa***	4011.8 ± 1278.0 **Aa**	4.7 ± 1.8 **ABa***
		STEM	40603.3 ± 1927.1 **Aa**	6120 ± 438.6 **ABa**	1621.8 ± 78.8 **Aa**	415.8 ± 37.6 **Aa**	1170.2 ± 478.9 **Aa**	6865.9 ± 242.3 **Aa**	24.4 ± 4.3 **ABa***
		LEAF	36693.3 ± 5883.6 **Aa**	7713.3 ± 2920.5 **Aa**	9869.3 ± 6190.6 **Aa***	162.1 ± 42.5 **Aa**	1182.8 ± 177.3 **Aa**	19924.6 ± 2576.3 **Aa***	121.8 ± 30.7 **ABa***
	H. courbaril	ROOT	7953.3 ± 5234.6 **Ba***	610 ± 480.4 **Ba**	643.9 ± 327.2 **Aa**	134.4 ± 61.9 **Aa**	555.5 ± 68.0 **Aa**	5008.0 ± 1889.1 **Aa**	2.7 ± 1.0 **ABb***
		STEM	20876.6 ± 3099.2 **Ca**	2410 ± 562.0 **BCb**	1793.5 ± 222.7 **Aa**	324.5 ± 80.1 **Aa**	1107.8 ± 582.9 **Aa***	1400.1 ± 459.3 **Aa**	14.9 ± 4.0 **ABb***
		LEAF	23640 ± 1509.9 **B**a	2683.3 ± 1105.4 **Bb**	1946.2 ± 500.8 **Ba**	265 ± 36.2 **Aa**	377.1 ± 71.5 **Ca**	7673.6 ± 704.1 **Bb**	78.6 ± 22.9 **Ab***
	H. stigonocarpa	ROOT	8033.3 ± 4254.7 **Ba**	2046.6 ± 1061.7 **Ba**	820.4 ± 512.4 **Aa**	352.2 ± 142.0 **Aa**	562.2 ± 96.0 **Aa**	10231.5 ± 7888.0 **Aa***	5.9 ± 3.0 **Bb**
		STEM	13783.3 ± 3362.3 **D**a	1173.3 ± 541.2 **Cb**	954.0 ± 462.4 **Aa**	135.7 ± 42.9 **Aa**	716.4 ± 256.9 **ABa**	161.5 ± 279.8 **Aa***	6.5 ± 1.8 **Bb**
		LEAF	22973.3 ± 5920.1 **Ba***	4220 ± 255.1 **ABb**	1674.3 ± 244.5 **Ba**	280.3 ± 30.7 **Aa**	472.6 ± 108.0 **Ba**	5923.5 ± 936.2 **Bb**	65.1 ± 38.9 **Bb***
	H. impetiginosus	ROOT	15103.3 ± 2578.8 **Aa***	6433.3 ± 3419.1 **Aa**	2661.7 ± 1070.9 **Aa**	953.6 ± 693.8 **Ab**	216.1 ± 162.6 **Aa***	5519.6 ± 1792.2 **Aa**	59.6 ± 36.2 **Aa**
		STEM	33976.6 ± 3241.6 **B**a	8090 ± 785.4 **Ab**	3882.8 ± 604.6 **Aa**	855.1 ± 342.8 **Aa**	527.8 ± 263.2 **Ba**	3177.1 ± 606.2 **Aa**	51.3 ± 17.1 **Aa**
		LEAF	39743.3 ± 3677.9 **Aa**	6973.3 ± 1459.4 **Ab**	3992.5 ± 262.3 **Ba**	647.3 ± 140.1 **Aa**	884.3 ± 429.0 **ABa***	17662.9 ± 1390.4 **Ab***	91.2 ± 4.8 **Aa**
RS	*E. contortisiliquum*	ROOT	1380 ± 1140.7 **Aa***	6716.6 ± 2722.6 **Aa**	558.1 ± 238.1 **Aa**	441.6 ± 69.1 **Ba**	97.1 ± 29.3 **Ab**	6785.1 ± 1899.5 **Aa**	9.7 ± 2.0 **ABa***
		STEM	12960 ± 2012.1 **Ab**	6260 ± 1199.2 **Ba**	825.2 ± 95.2 **Aa**	454.8 ± 111.6 **Aa**	13.2 ± 22.8 **Ab***	3473.3 ± 819.7 **Aa**	26.6 ± 2.2 **ABa***
		LEAF	8880 ± 3264.4 **Ab**	8260 ± 1390.1 **Ca**	2909.7 ± 384.0 **Ab**	1110.1 ± 1460.9 **Aa**	514.4 ± 135.0 **Ab***	14593.4 ± 10259.8 **Ba***	129.1 ± 38.9 **ABa***
	H. courbaril	ROOT	943.3 ± 473.7 **Ab**	2766.6 ± 1879.9 **Ba***	290.9 ± 245.5 **Aa**	721.2 ± 309.3 **Ba**	364.2 ± 229.5 **Aa**	8993 ± 7343.6 **Aa**	28.1 ± 9.2 **ABa***
		STEM	4656.6 ± 1048.4 **Bb**	10423.3 ± 1591.6 **Aa**	1078.0 ± 516.8 **Aa**	759.8 ± 267.7 **Aa**	232.5 ± 28.0 **Ab***	5977.3 ± 1738.0 **Aa**	140.9 ± 45.3 **ABa***
		LEAF	3510 ± 365.1 **Ab**	13320 ± 2785.3 **Ba**	290.9 ± 95.2 **Aa**	242.0 ± 53.5 **Aa**	712.6 ± 136.0 **Aa***	22186.3 ± 3024.8 **ABa***	455.4 ± 40.3 **ABa***
	H. stigonocarpa	ROOT	2180 ± 2321.2 **Ab**	3656.6 ± 1803.2 **ABa**	319.5 ± 140.4 **Aa**	805.3 ± 420.1 **Ba**	179.5 ± 108.9 **Ab***	6731.2 ± 1298.2 **Aa**	23.5 ± 7.8 **Aa**
		STEM	5606.6 ± 1654.0 **Bb**	4350 ± 727.4 **Ba**	586.7 ± 243.2 **Aa**	399.9 ± 19.2 **Aa**	305.3 ± 142.4 **Ab**	4792.6 ± 2722.9 **Aa**	109.0 ± 38.7 **Aa**
		LEAF	4220 ± 1787.8 **Ab**	8643.3 ± 2583.1 **Ca**	281.4 ± 205.5 **Aa**	328.3 ± 67.5 **Aa**	658.6 ± 137.1 **Aa***	24717.2 ± 3694.5 **Aa***	463.7 ± 300.4 **Aa***
	H. impetiginosus	ROOT	4183.3 ± 1058.7 **Ab**	7526.6 ± 2016.2 **Aa***	1078.0 ± 132.9 **Aa**	7935.9 ± 4202.8 **Aa***	355.9 ± 129.1 **Aa**	7754.4 ± 370.1 **Aa**	44.4 ± 13.4 **Aa**
		STEM	9240 ± 524.5 **ABb**	11740 ± 1768.0 **Aa***	1464.4 ± 414.0 **Ab**	1146.1 ± 549.3 **Aa**	163.9 ± 107.1 **Ab**	7215.9 ± 6291.3 **Aa**	63.8 ± 12.7 **Aa**
		LEAF	8723.3 ± 559.4 **Ab**	17886.6 ± 1630.8 **Aa***	820.4 ± 342.9 **Ab**	438.6 ± 29.3 **Aa**	598.0 ± 38.0 **Aa**	28459.8 ± 2970.4 **Aa***	121.3 ± 37.0 **Aa**

Ca: Calcium; K: Potassium; Mg: Magnesium; Al: Aluminum; P: Phosphorus; N: Nitrogen; and Mn: Manganese.

Significant differences and interactions ($p<0.05$): species:substrat:tissue.

Capital letters indicate differences between species, in the same tissue and substrate, in each response variable.

Lowercase letters indicate differences between substrate, in the same tissue and species

* Indicate differences between tissues, in the same species and substrate

Table 4: Mean and standard deviation of the translocation factor of nutrients and metals by species in each substrate (LOT= Lithium ore tailings and RS= Reference Soil). Nutrient or metal content is given per 1g dry weight of the sample. All values are means of triplicates ± SD.

SUBSTRATE	SPECIES	Translocation Factor (TF)							
		Ca	K	Mg	Al	P	N	Mn	Li
LOT	*E. contortisiliquum*	46.00 ± 16.00 **A**	2.09 ± 0.55	35.80 ± 17.80 **Aa**	2.21 ± 1.10	5.12 ± 0.35	7.00 ± 1.54 **Aa**	33.00 ± 10.50 **A**	4.53 ± 2.62
	H. courbaril	9.20 ± 8.52 **B**	35.60 ± 53.40	7.01 ± 3.54 **Bb**	5.15 ± 2.85	2.80 ± 1.55	1.96 ± 0.60 **Bb**	39.00 ± 21.60 **A**	4.81 ± 1.26
	H. stigonocarpa	6.73 ± 6.22 **B**	3.18 ± 1.58	6.07 ± 6.87 **Ba**	1.35 ± 0.63	2.12 ± 0.17	0.85 ± 0.51 **Bb**	16.50 ± 12.70 **AB**	0.88 ± 1.05
	H. impetiginosus	5.00 ± 1.04 **B**	2.80 ± 1.51	3.29 ± 1.32 **Ba**	1.99 ± 0.86	10.40 ± 9.49	3.99 ± 1.02 **Aba**	2.92 ± 1.36 **B**	0.97 ± 0.38
RS	*E. contortisiliquum*	33.40 ± 37.10 **A**	2.61 ± 1.68	7.59 ± 3.16 **Ab**	3.50 ± 3.03	5.64 ± 1.33	3.01 ± 2.09 **Ab**	17.00 ± 7.50 **A**	52.90 ± 61.80
	H. courbaril	10.80 ± 6.53 **B**	10.50 ± 4.21	14.60 ± 19.00 **Aa**	1.46 ± 0.26	3.67 ± 2.62	4.42 ± 2.39 **Aa**	22.40 ± 5.82 **A**	4.58 ± 1.83
	H. stigonocarpa	17.80 ± 24.10 **B**	4.23 ± 2.13	3.58 ± 3.04 **Ab**	1.06 ± 0.48	6.55 ± 2.92	4.51 ± 1.04 **Aa**	25.40 ± 12.30 **AB**	3.76 ± 1.91
	H. impetiginosus	4.44 ± 0.85 **B**	4.10 ± 0.92	2.16 ± 0.70 **Ab**	0.22 ± 0.06	2.34 ± 0.82	4.62 ± 0.92 **Aa**	4.56 ± 2.08 **B**	1.12 ± 0.89

Ca: Calcium; K: Potassium; Mg: Magnesium; Al: Aluminum; P: Phosphorus; N: Nitrogen; Li: Lithium and Mn: Manganese.

Significant differences and interactions ($p<0.05$): Ca = species; Mn = species; Mg = species:substrate; and N = species:substrate. No significant: K, Al, P, Li

Capital letters indicate differences between species in each substrate, in each response variable.

Lowercase letters indicate differences between substrate in each species

Biometric indices, biomass production, and tolerance indices

Handroanthus impetiginosus had higher SLA and LAR than the other species in both substrates (Fig. 3a, c, interaction between species and substrate). Also, *E. contortisiliquum* showed higher SLA and LAR in LOT than in RS, and *H. impetiginosus* showed higher LAR in RS than in LOT (Fig. 3a, c, interaction between substrate and species). *Hymenaea courbaril* and *H. stigonocarpa* exhibited the highest ALA on both substrates. However, only *E. contortisiliquum* and *H. impetiginosus* demonstrated higher ALA in RS than in LOT (Fig. 3b, interaction between substrate and species).

Hymenaea stigonocarpa and *H. courbaril* showed higher LDM in LOT than other species (Fig. 2d, interaction between species and substrate). However, *E. contortisiliquum* and *H. impetiginosus* showed lower LDM in LOT than RS (Fig. 3d, interaction between substrate and species). *Enterolobium contortisiliquum* had higher RDM and TDM than the other species in the RS, while *H. impetiginosus* had lower RDM and TDM in both substrates (Fig. 3e, f, interaction between species and substrate). The LOT decreased RDM and TDM in *E. contortisiliquum*, *H courbaril*, and *H. stigonocarpa* (Fig. 3e, f, interaction between substrate and species). *Hymenaea courbaril* invested more in root than in stem in LOT (highest RSR), while *E. contortisiliquum* had the highest RSR in the RS (Fig. 3g, interaction between species and substrate). Only *E. contortisiliquum* showed a higher RSR in RS than in LOT (Fig. 3g, interaction between substrate and species). The species had higher TLA, SDM, SL, and RL in RS than in the LOT (Fig. S5a-d, simple effect of substrate).

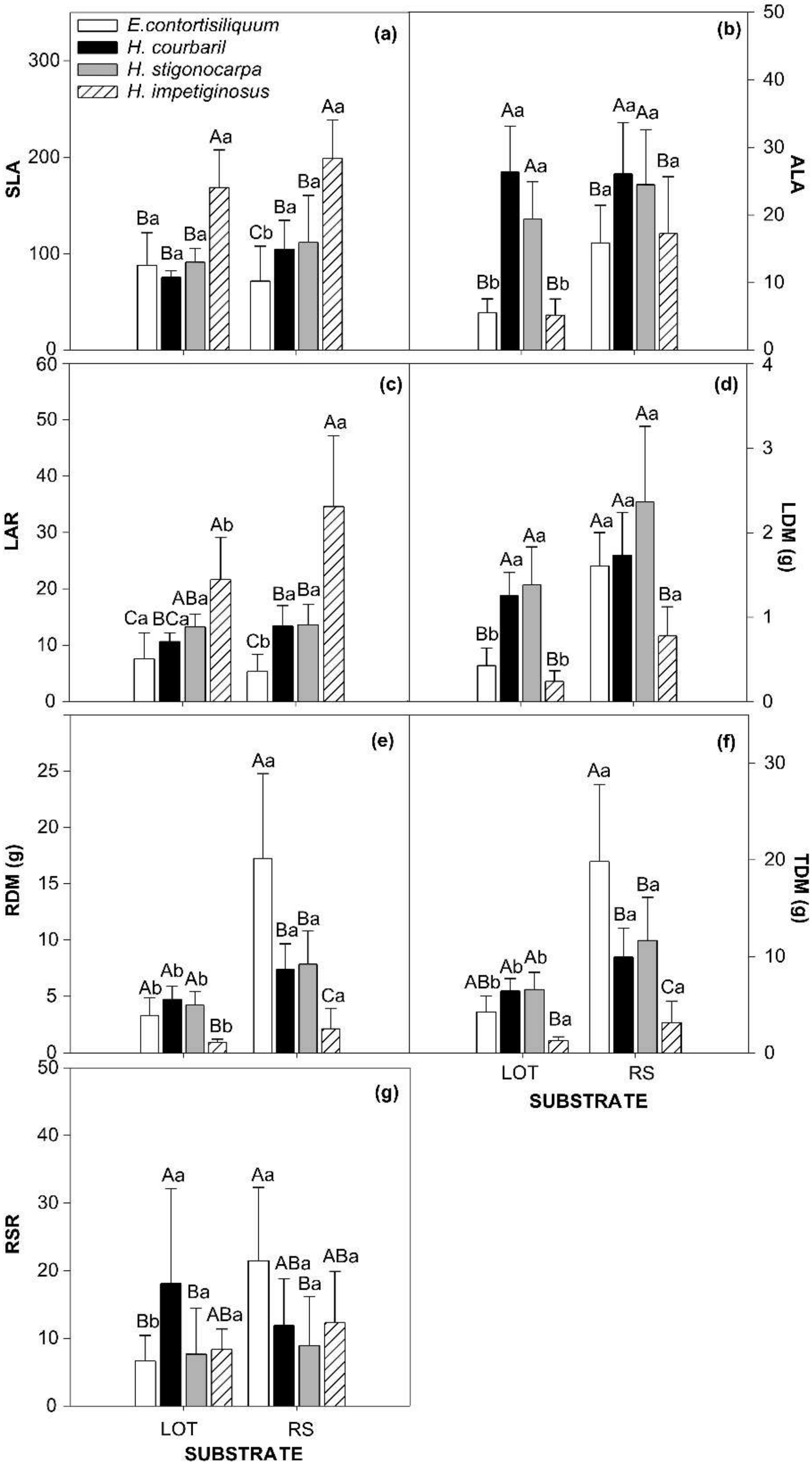

Fig. 3 Morphological characteristics and biometric indices of four woody species growing in Lithium ore tailings (LOT) and reference soil (RS) in open chambers. (a) SLA = specific leaf area, (b) ALA = average leaf area, (c) LAR = leaf area ratio, (d) LDM = leaf dry mass, (e) RDM = root dry mass, (f) TDM = total dry mass, (g) RSR = root/shoot ratio. Bars represent means and standard deviation. Capital letters compare differences between species within the same substrate. Small letters compare differences of the same species in different substrate (double interaction between species and substrate, $p<0.05$).

The STI did not vary between species (Fig. 4a). However, the LTI was highest in *H. courbaril* and lowest in *E. contortisiliquum* (Fig. 4b). None of the species reached ITT values > 1, with *E. contortisiliquum* presenting the lowest RTI and TTI values for LOT (Fig. 4c, d).

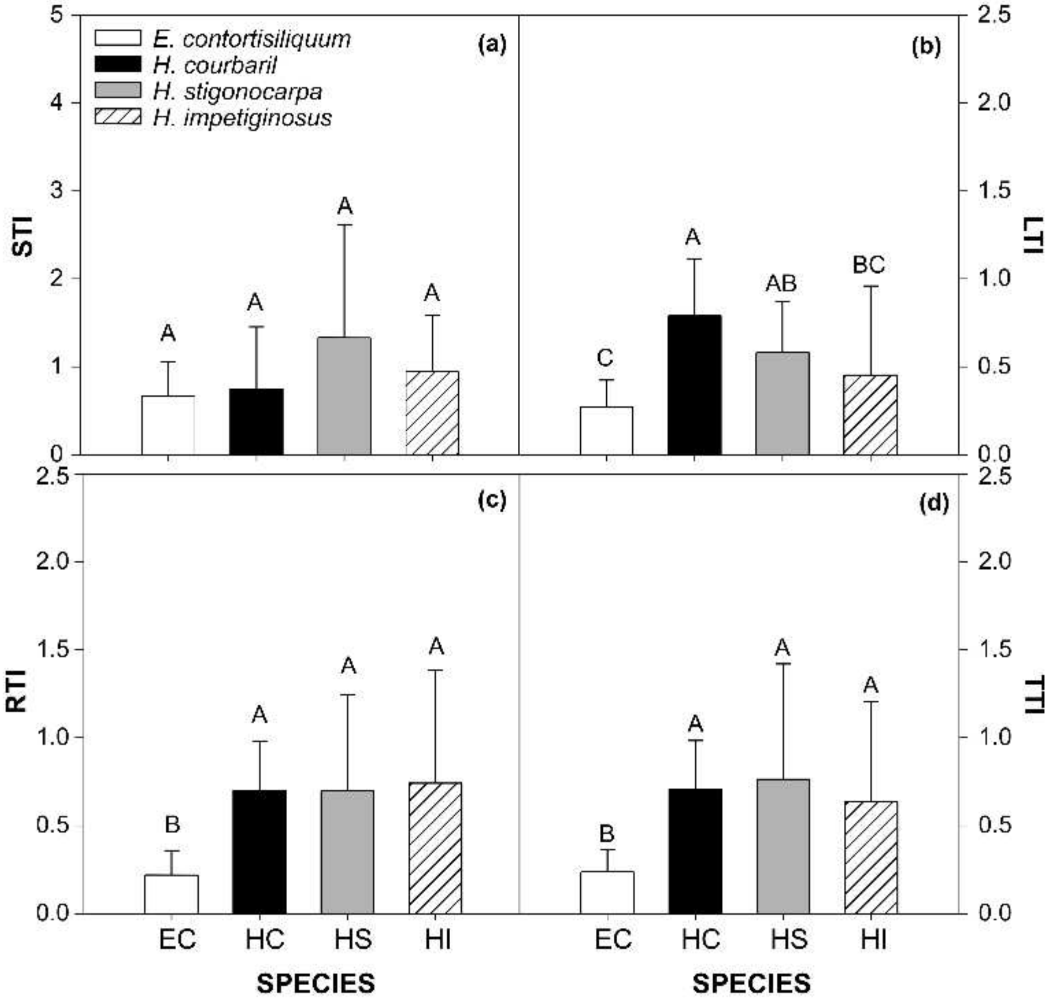

Fig. 4 Tolerance Index of four woody species growing on Lithium ore tailings (LOT) and reference soil (RS) in open chambers. (a) STI = stem tolerance indices, (b) LTI = leaf tolerance indices (c) RTI = root tolerance indices, and (d) TTI = total tolerance indices (stem + leaf + root). Capital letters compare differences between species ($p<0.05$).

Discussion

Photosynthetic parameters

The reduction in F_v/F_m is an indicator of stress in plants, signaling a possible decrease in photosynthetic capacity due to stress or damage to the photosynthetic apparatus, such as a decrease in light absorption or oxidation of PSII reaction centers (Baker and Rosenqvist 2004; Ribeiro et al., 2005). The low efficiency in light conversion to photosynthesis, associated with the low total and b chlorophyll content in plants grown in LOT, suggests a lower repair capacity of PSII (Allakhverdiev and Murata, 2004). The decrease in chlorophyll levels in plants grown in LOT affected F_v/F_m, suggesting that the species had photosynthetic activity impaired in LOT. The reduction in chlorophyll levels indicates a progressive decrease in the photosynthetic capacity of plants (Rios et al., 2023). In contrast, keeping ϕPSII and ETR (even with low F_v/F_m values) suggests an adjustment in plants that face unfavorable conditions. Under stress, plants may adjust their ϕPSII to optimize the use of available light energy, thereby compensating for the reduction in maximum PSII efficiency and PSII repair capacity (Allakhverdiev, 2011). However, plants grown in LOT and soil did not change NPQ values. A lower potential efficiency added to the non-increase of NPQ in the LOT may indicate an even greater loss, as the energy will not be converted or re-emitted in the form of heat, which could damage the photosystems (Allakhverdiev, 2011). Although effective efficiency and ETR were not affected, over time the damage to plants in LOT may be more significant.

Vegetative growth

The higher early leaf production in *E. contortisiliquum* in LOT associated with the shorter leaf lifespan, may be related to the strategy of optimizing the use of initial resources. *Enterolobium contortisiliquum* is a species known for its fast growth (Lorenzi, 2002; Brack and Grings et al., 2011), which explains its quick initial leaf production compared to other species. While the increase in leaf production initially was not sustained over time in LOT. The decreased leaf production in *E. contortisiliquum* could be due to its low tolerance to LOT. The slower leaf production in *H. impetiginosus*, *H. courbaril*, and *H. stigonocarpa* may be associated with the slower growth of the species (Reich, 2014). The lower leaf production over time, higher leaf expansion rate, and longer leaf life span in *H. courbaril* and *H. stigonocarpa* may indicate an optimization of resource use over time due to greater longevity and allocation of leaf biomass (Ruan et al., 2013). This resource optimization is more evident for *H. stigonocarpa*, which did not have leaf production affected over time by LOT. The absence of a negative effect of LOT on stem diameter of all species may be associated with the experimental period (it was not possible to observe great secondary development of the species), since this attribute presents less plasticity concerning leaves (Costa et al., 2022).

Mineral nutrition

The higher [Li] in plants grown in the LOT aligns with high Li availability in LOT. Herbaceous C_3 plants have demonstrated toxic signs at concentrations > 50 mg/kg^{-1}, such as growth restriction, decreased aerial biomass production, and leaf area reduction (in non-bioaccumulating plants, Shahzad et al., 2016). Overall, the [Li] in plants may vary between 0.2 and 30 mg/kg^{-1} (Shakoor et al., 2023b). Therefore, the [Li] found in *H. impetiginosus*, *E. contortisiliquum*, and *H. stigonocarpa* plants (> 30 mg/kg^{-1}) could have negatively affected these plants. Even accumulating a high Li amount, *H. stigonocarpa* and *H. impetiginosus* did not translocate Li. The Li accumulation in roots could be less harmful to *H. stigonocarpa*, but *H. impetiginosus* shows a high reduction in biomass allocation in roots. However, the effects were even more pronounced in *E. contortisiliquum*, in which individuals on LOT presented harmful effects in leaf and root growth. Some authors (Aral e Vecchio-Sadus, 2011; Shahzad et al., 2016; Shakoor et al., 2023a) reported that high Li accumulation could reduce plant dry weight, being toxic to the plants. On the other hand, *H. courbaril* did not accumulate a high amount of Li in any tissue analyzed, showing that this plant species could present some mechanism that prevents Li absorption in the soil, as exudate of some secondary product of photosynthesis, as organic acids (Wang et al., 2017; Bortoloti and Baron, 2022).

Generally, adding silicates (eg. Ca and Mg) in acidic soils could correct the acidity and increase the availability of Ca, Mg, and P (Alcarde, 1992; Maio et al., 2011; Freitas et al., 2015). LOT is within the silicate group (Castro et al., 2016), and this may also have influenced the lower availability of Al and Mn in their soluble form due to the increase in pH (Freitas et al., 2015). Although in LOT the species were able to absorb Al and Li, it favored the increased absorption and accumulation of Ca, Mg (in *E. contortisiliquum* and *H. impetiginosus*), and P but decreased the accumulation of K and N in the leaves of *H. stigonocarpa*, *H. courbaril*, and *H. impetiginosus*. Hayyat et al. (2020) discuss that higher [Li] in the substrate may decrease the availability of K, Na, and N for plants. Though Ca and Mg are in higher concentrations in the LOT than in the soil, their concentration and accumulation in plants are within levels suitable for growth (10-50g/kg^{-1} and 500mg/kg^{-1}, respectively, Furlani 2004; Alejandro, 2020). On the other hand, the high accumulation of Mg in the leaves of *E. contortisiliquum* exceeded typical values for plants >5g/kg^{-1} (Dechen and Nachtigall, 2007; Broadley et al., 2012), possibly triggering harmful metabolic processes. Although Mg toxicity is commonly associated with necrotic spots on veins or leaves (Verbruggen and Hermans, 2013), other metabolic processes have been demonstrated, such as changes in carbon fixation and transfer, and an increase in reactive oxygen species (Guo et al. al., 2015). Therefore, the reduction in chlorophyll (b and total) in *E. contortisiliquum* may be associated with Mg toxicity, compromising plant growth.

The lower absorption of K by plants in LOT may influence some protein functions, the osmotic balance of cells, and the opening of stomata (White and Karley, 2010). Under K-limiting conditions, the plant tends to redistribute K from older tissues to young ones, with translocation from the vacuole to the cytosol and plastids of great importance (Behr et al., 2022). However, in the vacuole, K may be partially replaced by Na to maintain osmotic balance (White et al., 2021). In LOT, even with lower absorption of K, we can observe high levels of Na, which possibly attenuated physiological processes that would be triggered by K deficiency (e.g. the accumulation of putrescine due to the reduction of protein synthesis and accumulation of amino acids, Pi et al., 2016; Wakeel et al., 2010).

Biomass production, tolerance, and survivorship

The higher LAR and SLA in *E. contortisiliquum* indicate the production of thinner leaves in plants grown in the LOT, which explains its lower LDM in plants grown in this substrate. Castro et al. (2016) also observed lower biomass despite the initial increase in the number of leaves in *Corymbia citriodora* Hook. in a substrate with the addition of LOT. Furthermore, the lower biomass and higher SLA are similar responses to the toxicity of woody plants growing in mining tailings (Cruz et al., 2022). *Enterolobium contortisiliquum* and *H. impetiginosus* exhibited thinner and less durable leaves than *H. courbaril* and *H. stigonocarpa*, which maintained their leaves until the end of the experiment, which contributed to a higher LDM in these species. Leaf longevity is related to photosynthetic gain and leaf maintenance cost (Kikuzawa, 1991, Wright et al., 2004; Díaz et al., 2016). *Hymenaea courbaril* and *H. stigonocarpa* probably invest in a low SLA as a strategy to optimize resource use, reducing foliar production costs by keeping their leaves longer (Kikuzawa, 1988, Wright et al., 2004). This strategy confers an advantage in stressful environments due to the resource use optimization of nutrients (Garnier and Poorter, 2007; Poorter et al., 2011; Pérez-Harguindeguy et al., 2013).

Enterolobium contortisiliquum plants grown in LOT showed a greater investment in shoot than in root growth (low RSR in LOT). Although the RSR in *H. courbaril*, *H. stigonocarpa*, and *H. impetiginosus* plants did not differ between substrates, the sum of the total dry mass of these species was significantly lower in LOT. LOT possibly limits root development due to nutritional deficiency caused by high pH, high concentration of Li, Ca, Mg, and Fe deficiency, added to physical properties such as a high proportion of silt, which facilitates compaction and limits the formation of aggregates due to lack of cohesion, characteristics found in the tailings (Esteves et al., 2020; Zanchi et al., 2022). Although the LOT has a high saturation and sum of bases, an indicator of high fertility, this result is mainly due to the high Ca and Mg concentration. Furthermore, the high pH of LOT contributes to its nutritional deficiency, as an alkaline pH limits efficient nutrient absorption by plants (Andrade et al., 2018; Zanchi et al., 2022), reducing plant development in LOT. Also, compaction facilitated by physical properties such as a silty texture, high density, and low porosity (Nardi et al., 2002; Lier and Van., 2010; Andrade et al., 2018; Matos et al., 2020) may exert physical resistance to the roots (Esteves et al., 2020) which, together with the decrease in oxygen, could result in root hypoxia hindering root development (Bengough et al., 2006). In the long term, the limitations imposed on root growth slow down the development of shoot tissues in plants due to decreased ability to acquire resources (Gagnon et al., 2020; Esteves et al., 2020).

The change in plant biomass has been used as an indicator of the tolerance of plants to stressful environments such as soils contaminated by metals (Meyer et al., 2016; Shi et al., 2011; Nautiyal et al., 2002). Contrary to what we expected, *E. contortisiliquum* (wide geographic distribution) was the species with the lowest biomass production when grown in LOT, mainly in leaves and roots (lowest RTI and TTI), being considered less tolerant to LOT. Although *E. contortisiliquum* presents a wide geographical distribution and is considered potentially tolerant and able of bioaccumulating metals such as Cu, Zn, Ca, Cr, and Ni (Silva et al., 2011; Silva et al., 2018; Zanchi et al., 2022), in our study, this species demonstrated low tolerance to LOT, due to the decreased biomass production. The fast emergence, growth, and acquisition of initial resources by *E. contortisiliquum* may have favored a greater early leaf development, but fast leaf abscission together with the limitation of root growth by LOT did not guarantee the maintenance of leaf production in the long term. Despite the higher biomass production of other species than *E. contortisiliquum*, none reached a TI > 1, indicative of species considered tolerant (Meyer et al., 2016). The other species also showed biomass losses in stems, leaves, and roots, but managed to maintain their mass production like those grown in soil, mainly *H. courbaril* and *H. stigonocarpa*, demonstrating greater tolerance of these species to LOT. The similar area and dry mass of leaves and RSR in *H. courbaril* and *H. stigonocarpa* plants grown in LOT and soil supports this result.

When evaluating the phytoremediation potential of species, a metal translocation from roots to shoots > 1 indicates metal phytoextraction capacity (Ghosh and Singh, 2005; Rios et al., 2023). Although all species present high TF for the macronutrients Ca, K, Mg, P, and N, and micronutrient Mn, only *E. contortisiliquum* and *H. courbaril* showed high Li transfer in the LOT, not limiting the accumulation only to their roots. *Hymenaea courbaril* due to the lower loss of biomass than *E. contortisiliquum* and the translocation of Li could be considered a promising option for phytoextraction, since the metals are transferred to the aerial part, allowing easier removal (Yoon et al., 2006). While *H. stigonocarpa* could be indicated as a bio-accumulator of Li in roots, without apparent effects of LOT on leaf production. Furthermore, different from our predictions, species tolerance is not associated with the geographic distribution in which they occur. Our results indicate that species with more restricted geographical distribution, such as *H. courbaril* and *H. stigonocarpa* (Carvalho et al., 2006; Nascimento et al., 2011; Rosado et al., 2023), were more tolerant to LOT than other more widely distributed species.

Our results indicate that plants grown on LOT invested more in survival than growth. This response may be associated with ecological strategy in the acquisition and conservation of resources (Reich, 2014), indicating a trade-off between growth and survival. Negreiros et al. (2016) found evidence of compensation mechanisms for shrub species in nutritionally limiting environments with high light availability. Generally, fast-growing species with less durable structure (such as leaves) and higher growth rates use resources more quickly (acquisitive), while slow-growing species retain foliar nutrients by keeping their leaves longer (conservative, Wright et al. 2004; Boonman et al., 2020; Guimarães et al., 2022). Our results suggest that *E. contortisiliquum* exhibited typical characteristics of an acquisitive strategy in investing to quickly obtain resources. On the other hand, *H. courbaril* and *H. stigonocarpa* showed characteristics of a conservative strategy, suggesting a more efficient and long-lasting allocation of resources to optimize long-term survival. For *H. impetiginosus*, we have not identified a specific ecological strategy. These patterns are in line with previous evidence suggesting that fast-growing species tend to adopt acquisitive strategies, while slow-growing species adopt more conservative strategies in resource use.

Conclusion

LOT results in distinct effects on the development of the woody species studied. Although all species present possible photosynthetic adjustments, maintaining ϕPSII and ETR levels, ensuring survival, failure to increase NPQ does not guarantee long-term photosynthetic efficiency in LOT. The LOT decreased the length and biomass of the stem and root of the studied species. LOT favored the accumulation of Mg and reduced K absorption, possibly causing Mg toxicity in *E. contortisiliquum*, affecting metabolic processes such as photosynthesis. Despite the lower absorption of K, the high levels of Na in the LOT possibly attenuated physiological processes that would be triggered by K deficiency. Also, Li accumulation in the shoots of *E. contortisiliquum* may harm leaf and root production, but Li accumulation in roots could not be toxic to *H. stigonocarpa*. *Enterolobium contortisiliquum* presents the lowest tolerance to LOT, with lower biomass production, while *H. courbaril* and *H. stigonocarpa* were the most tolerant. This result suggests that the tolerance to Li is not related to the species's geographic distribution. Furthermore, *H. courbaril* demonstrated FT>1, making it a promising alternative for Li phytoextraction. Our results offer insights into the impacts of LOT on the early development of woody species, as well as on the selection of species with phytoremediation potential. Therefore, the results found in this study show that functional traits based on growth and photosynthetic activities are good indicators of species tolerance under tailings stress.

Statements & Declarations:

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-Ethical Approval

Not applicable

-Consent to Participate

The authors declare to be aware of the participation this manuscript

-Consent to Publish

The authors declare to be aware of the publish this manuscript

-Authors Contributions

Maria Gracielle Rodrigues Maciel: Investigation, Conceptualization, Data acquisition (field work), Data analysis, Writing- Original draft preparation, Visualization, Writing - Review and Editing, Project administration.
Nayara Magry Jesus Melo: Investigation, Conceptualization, Data acquisition (field work), Data analysis, Review.
Gustavo Júnio Santos Oliveira: Data acquisition (field work).
Marihus Altoé Baldotto: Review.
João Paulo Souza: Conceptualization, Data analysis, Funding acquisition, Supervision, Project administration, Visualization, Writing - Review and Editing.

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-Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

-Data Availability

Our dataset is available and may be requested from the corresponding author.

References

Agusdinata, D.B., Liu, W., Eakin, H., Romero, H., 2018. Socio-environmental impacts of lithium mineral extraction: towards a research agenda. Environ. Res. Lett. 13, 123001. https://doi.org/10.1088/1748-9326/aae9b1

Alcarde, J. C, 1992. Soil acidity correctors: characteristics and technical interpretations. São Paulo: ANDA, (Technical Bulletin, 6). (in Portuguese)

Alejandro, S., Höller, S., Meier, B., Peiter, E., 2020. Manganese in Plants: From Acquisition to Subcellular Allocation. Front. Plant Sci. 11, 300. https://doi.org/10.3389/fpls.2020.00300

Allakhverdiev, S.I., 2011. Recent progress in the studies of structure and function of photosystem II. Journal of Photochemistry and Photobiology B: Biology 104, 1–8. https://doi.org/10.1016/j.jphotobiol.2011.03.010

Allakhverdiev, S.I., Murata, N., 2004. Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage–repair cycle of Photosystem II in Synechocystis sp. PCC 6803. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1657, 23–32. https://doi.org/10.1016/j.bbabio.2004.03.003

Andrade, G.F., Paniz, F.P., Martins, A.C., Rocha, B.A., Da Silva Lobato, A.K., Rodrigues, J.L., Cardoso-Gustavson, P., Masuda, H.P., Batista, B.L., 2018. Agricultural use of Samarco's spilled mud assessed by rice cultivation: A promising residue use? Chemosphere 193, 892–902. https://doi.org/10.1016/j.chemosphere.2017.11.099

Anjum, S.A., Ashraf, U., Khan, I., Tanveer, M., Ali, M., Hussain, I., Wang, L.C., 2016. Chromium and Aluminum Phytotoxicity in Maize: Morpho-Physiological Responses and Metal Uptake. CLEAN Soil Air Water 44, 1075–1084. https://doi.org/10.1002/clen.201500532

Aral, H., Vecchio-Sadus, A., 2008. Toxicity of lithium to humans and the environment—A literature review. Ecotoxicology and Environmental Safety 70, 349–356. https://doi.org/10.1016/j.ecoenv.2008.02.026

Aral, H., Vecchio-Sadus, A., 2011. Lithium: Environmental Pollution and Health Effects, in: Encyclopedia of Environmental Health. Elsevier, pp. 116–125. https://doi.org/10.1016/B978-0-444-63951-6.00531-3

Baker, N.R., 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. Journal of Experimental Botany 55, 1607–1621. https://doi.org/10.1093/jxb/erh196

Behr, J.H., Zörb, C., 2023. Interactive effect of lithium on concentration of alkali cations in sugar beet (*Beta vulgaris* L.) under saline conditions. J. Plant Nutr. Soil Sci. 186, 38–49. https://doi.org/10.1002/jpln.202200079

Bengough, A.G., Bransby, M.F., Hans, J., McKenna, S.J., Roberts, T.J., Valentine, T.A., 2006. Root responses to soil physical conditions; growth dynamics from field to cell. Journal of Experimental Botany 57, 437–447. https://doi.org/10.1093/jxb/erj003

Boonman, C.C.F., Van Langevelde, F., Oliveras, I., Couédon, J., Luijken, N., Martini, D., Veenendaal, E.M., 2020. On the importance of root traits in seedlings of tropical tree species. New Phytologist 227, 156–167. https://doi.org/10.1111/nph.16370

Bortoloti, G.A., Baron, D., 2022. Phytoremediation of toxic heavy metals by Brassica plants: A biochemical and physiological approach. Environmental Advances 8, 100204. https://doi.org/10.1016/j.envadv.2022.100204

Bovet, L., Kammer, P.M., Meylan-Bettex, M., Guadagnuolo, R., Matera, V., 2006. Cadmium accumulation capacities of Arabis alpina under environmental conditions. Environmental and Experimental Botany 57, 80–88. https://doi.org/10.1016/j.envexpbot.2005.04.010

Brack, P., Grings, M. 2011. *Enterolobium contortisiliquum*, in: Coradin, L., Siminski, A., Reis, A., Brazil (Eds.), Native species of Brazilian flora with current or potential economic value: plants for the future - South Region, Biodiversity. Ministério do Meio Ambiente, Brasília, DF. Cap. 5, pp. 457-460. (in Portuguese)

Braga, P. F. A.; França S. C. A. 2013. Series studies and documents. Lithium: a strategic mineral, 81st ed, IV. CETEM/MCTI, Rio de Janeiro. (in Portuguese)

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F., 2012. Function of Nutrients, in: Marschner's Mineral Nutrition of Higher Plants. Elsevier, pp. 191–248. https://doi.org/10.1016/B978-0-12-384905-2.00007-8

Carlucci, M.B., Brancalion, P.H.S., Rodrigues, R.R., Loyola, R., Cianciaruso, M.V., 2020. Functional traits and ecosystem services in ecological restoration. Restoration Ecology 28, 1372–1383. https://doi.org/10.1111/rec.13279

Carmo, C. D. S., De Araujo, W. S., Bernardi, A. D. C., Saldanha, M. F. C., 2000. Methods for analyzing plant tissues used at Embrapa Solos, 21st ed. Embrapa Solos, Rio de Janeiro. Available in: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/62212/1/Metodo-de-analise-de-tecido.pdf.(in Portuguese)

Carvalho, P. E. R. 2006. Brazilian tree species collection. V. 2. Brasília, DF: Embrapa Informação Tecnológica; Colombo, PR : Embrapa Florestas. (in Portuguese)

Castro, E.B., Santos, L.D.T., Fernandes, L.A., Tajima, C.Y., 2016. Use of Aluminum Silicate Partial Substitution in the Substrate for Seedlings Production *Corymbia citriodora*. Floresta Ambient. 23, 229–236. https://doi.org/10.1590/2179-8087.106814 (in Portuguese)

Costa, J.P., Dias Neto, O.C., Santos, L.C.S., Rodrigues, A.W., Rios, J.M., Prado-Júnior, J.A., Vale, V.S.D., 2022. Functional traits of cerrado *sensu stricto* arbored species and its importance for the maintenance of native communities. Ciênc. Florest. 32, 1807–1829. https://doi.org/10.5902/1980509842472

Cruz, F.V.D.S., Gomes, M.P., Bicalho, E.M., Garcia, Q.S., 2022. Fertilization assures mineral nutrition but does not overcome the effects of Fe accumulation in plants grown in iron ore tailings. Environ Sci Pollut Res 29, 18047–18062. https://doi.org/10.1007/s11356-021-16989-3

Dechen, A. R., Nachtigall, G. R, 2007. Elements required for plant nutrition. Soil fertility, Viçosa: Brazilian Soil Science Society, pp. 91-132. (in Portuguese)

Díaz, S., Kattge, J., Cornelissen, J.H.C., et al. 2016. The global spectrum of plant form and function. Nature 529, 167–171. https://doi.org/10.1038/nature16489

Donagema, G.K., Campos, D.V.B. Calderano, S.B., Teixeira, W.G., Viana, J.H.M., 2011. Manual of soil analysis methods, 2nd ed. Rio de Janeiro: Embrapa Soils. (in Portuguese)

Elektorowicz, M., Keropian, Z., 2015. Lithium, Vanadium and Chromium Uptake Ability of *Brassica juncea* from Lithium Mine Tailings. International Journal of Phytoremediation 17, 521–528. https://doi.org/10.1080/15226514.2013.876966

Esteves, G.D.F., De Souza, K.R.D., Bressanin, L.A., Andrade, P.C.C., Veroneze Júnior, V., Dos Reis, P.E., Da Silva, A.B., Mantovani, J.R., Magalhães, P.C., Pasqual, M., De Souza, T.C., 2020. Vermicompost improves maize, millet and *sorghum* growth in iron mine tailings. Journal of Environmental Management 264, 110468. https://doi.org/10.1016/j.jenvman.2020.110468

Ettler, V., Konečný, L., Kovářová, L., Mihaljevič, M., Šebek, O., Kříbek, B., Majer, V., Veselovský, F., Penížek, V., Vaněk, A., Nyambe, I., 2014. Surprisingly contrasting metal distribution and fractionation patterns in copper smelter-affected tropical soils in forested and grassland areas (Mufulira, Zambian Copperbelt). Science of The Total Environment 473–474, 117–124. https://doi.org/10.1016/j.scitotenv.2013.11.146

Freitas, L.B.D., Fernandes, D.M., Maia, S.C.M., 2015. Silicon on the mineral nutrition and aluminum accumulation in upland rice plants. Pesqui. Agropecu. Trop. 45, 440–448. https://doi.org/10.1590/1983-40632015v4538085 (in Portuguese)

Funk, J.L., Cleland, E.E., Suding, K.N., Zavaleta, E.S., 2008. Restoration through reassembly: plant traits and invasion resistance. Trends in Ecology & Evolution 23, 695–703. https://doi.org/10.1016/j.tree.2008.07.013

Furlani AMC, 2004. Nutrição mineral, in: Kerbauy G. B. (Ed.). Plant physiology. Rio de Janeiro: Guanabara Koogan pp. 40-75. (in Portuguese)

Gagnon, V., Rodrigue-Morin, M., Migneault, M., Tardif, A., Garneau, L., Lalonde, S., Shipley, B., Greer, C.W., Bellenger, J.-P., Roy, S., 2020. Survival, growth and element translocation by 4 plant species growing on acidogenic gold mine tailings in Québec. Ecological Engineering 151, 105855. https://doi.org/10.1016/j.ecoleng.2020.105855

Garnier, E., Poorter, H., 2007. Ecological Significance of Inherent Variation in Relative Growth Rate and Its Components, in: Valladares, F., Pugnaire, F. (Eds.), Functional Plant Ecology, Second Edition, Books in Soils, Plants, and the Environment. CRC Press. https://doi.org/10.1201/9781420007626.ch3

Ghosh, M., Singh, S.P., 2005. A Review On Phytoremediati On Of Heavy Metals And Utilization Of Its Byproducts. Appl Ecol Env Res 3, 1–18. https://doi.org/10.15666/aeer/0301_001018

Ginocchio, R., León-Lobos, P., Arellano, E.C., Anic, V., Ovalle, J.F., Baker, A.J.M., 2017. Soil physicochemical factors as environmental filters for spontaneous plant colonization of abandoned tailing dumps. Environ Sci Pollut Res 24, 13484–13496. https://doi.org/10.1007/s11356-017-8894-8

Gómez, L., Contreras, A., Bolonio, D., Quintana, J., Oñate-Sánchez, L., Merino, I., 2019. Phytoremediation with trees, in: Advances in Botanical Research. Elsevier, pp. 281–321. https://doi.org/10.1016/bs.abr.2018.11.010

Guimarães, Z.T.M., Dos Santos, V.A.H.F., Ferreira, M.J., 2022. Chlorophyll a fluorescence parameters are related to the leaf economics spectrum of tropical tree species in a mixed plantation. Trees 36, 763–775. https://doi.org/10.1007/s00468-021-02248-y

Guo, W., Chen, S., Hussain, N., Cong, Y., Liang, Z., Chen, K., 2015. Magnesium stress signaling in plant: Just a beginning. Plant Signaling & Behavior 10, e992287. https://doi.org/10.4161/15592324.2014.992287

Gupta, S., Nayek, S., Saha, R.N., Satpati, S., 2008. Assessment of heavy metal accumulation in macrophyte, agricultural soil, and crop plants adjacent to discharge zone of sponge iron factory. Environ Geol 55, 731–739. https://doi.org/10.1007/s00254-007-1025-y

Haridasan, M., Paviani, T.I., Schiavini, I., 1986. Localization of aluminium in the leaves of some aluminium-accumulating species. Plant Soil 94, 435–437. https://doi.org/10.1007/BF02374336

Hawrylak-Nowak, B., Kalinowska, M., Szymańska, M., 2012. A Study on Selected Physiological Parameters of Plants Grown Under Lithium Supplementation. Biol Trace Elem Res 149, 425–430. https://doi.org/10.1007/s12011-012-9435-4

Hayyat, M.U., Khan, A.U., Ali, S., Siddiq, Z., Sharif, F., 2020. Alleviation of lithium toxicity in *Sorghum* (*Sorghum vulgare* Pers.) by inoculation with lithium resistant bacteria. Appl. Ecol. Env. Res. 18, 7989–8008. https://doi.org/10.15666/aeer/1806_79898008

He, M., Zhang, K., Tan, H., Hu, R., Su, J., Wang, J., Huang, L., Zhang, Y., Li, X., 2015. Nutrient levels within leaves, stems, and roots of the xeric species Reaumuria soongorica in relation to geographical, climatic, and soil conditions. Ecology and Evolution 5, 1494–1503. https://doi.org/10.1002/ece3.1441

Heckenroth, A., Rabier, J., Dutoit, T., Torre, F., Prudent, P., Laffont-Schwob, I., 2016. Selection of native plants with phytoremediation potential for highly contaminated Mediterranean soil restoration: Tools for a non-destructive and integrative approach. Journal of Environmental Management 183, 850–863. https://doi.org/10.1016/j.jenvman.2016.09.029

Ilunga, Wa Ilunga, E., Mahy, G., Piqueray, J., Séleck, M., Shutcha, M.N., Meerts, P., Faucon, M.-P., 2015. Plant functional traits as a promising tool for the ecological restoration of degraded tropical metal-rich habitats and revegetation of metal-rich bare soils: A case study in copper vegetation of Katanga, DRC. Ecological Engineering 82, 214–221. https://doi.org/10.1016/j.ecoleng.2015.04.084

Kastori, R., Maksimović, I., Putnikdelić, M., 2022. Lithium in the Environment and its Effects on Higher Plants. Contemporary Agriculture 71, 226–239. https://doi.org/10.2478/contagri-2022-0030

Kikuzawa, K. 1988. Leaf Survivals of Tree Species in Deciduous Broad-Leaved Forests. Plant Species Biology, 3(2), 67–76. https://doi.org/10.1111/j.1442-1984.1988.tb00172.x

Kikuzawa, K., 1991. A Cost-Benefit Analysis of Leaf Habit and Leaf Longevity of Trees and Their Geographical Pattern. The American Naturalist 138, 1250–1263.

Laughlin, D.C., 2014. Applying trait-based models to achieve functional targets for theory-driven ecological restoration. Ecology Letters 17, 771–784. https://doi.org/10.1111/ele.12288

Lemougna, P.N., Yliniemi, J., Ismailov, A., Levanen, E., Tanskanen, P., Kinnunen, P., Roning, J., Illikainen, M., 2019. Recycling lithium mine tailings in the production of low temperature (700–900 °C) ceramics: Effect of ladle slag and sodium compounds on the processing and final properties. Construction and Building Materials 221, 332–344. https://doi.org/10.1016/j.conbuildmat.2019.06.078

Li, X., Gao, P., Gjetvaj, B., Westcott, N., Gruber, M.Y., 2009. Analysis of the metabolome and transcriptome of Brassica carinata seedlings after lithium chloride exposure. Plant Science 177, 68–80. https://doi.org/10.1016/j.plantsci.2009.03.013

Liberato, C. G., Barros, J. A. V. A., Virgilio, A., Machado, R. C., Nogueira, A. D. A., Nóbrega, J. A., Schiavo, D., 2017. Determination of macro and micronutrients in plants using the Agilent 4200 MP AES. Appl. Note Food Agric, 1-5.

Lier, Q. J. Van. 2010. Soil Physics. 1 ed. Viçosa: Brazilian Society of Soil Science, 298p. (in Portuguese)

Lorenzi, H. 2002. Brazilian trees: manual for identification and cultivation of tree plants native to Brazil. 4.ed. Nova Odessa: Plantarum Institute. 368p. (in Portuguese)

Lu, Y., Ranjitkar, S., Harrison, R.D., Xu, J., Ou, X., Ma, X., He, J., 2017. Selection of Native Tree Species for Subtropical Forest Restoration in Southwest China. PLoS ONE 12, e0170418. https://doi.org/10.1371/journal.pone.0170418

Maio, M.M., Sampaio, R.A., Nascimento, A.L., Prates, F.B.D.S., Rodrigues, M.N., Silva, H.P.D., Dias, A.N., Freitas, C.E.S., 2011. Physical attributes of soil fertilized with sewage sludge and with calcium and magnesium silicates. Rev. Ceres 58, 823–830. https://doi.org/10.1590/S0034-737X2011000600021 (in Portuguese)

Martin, G., Rentsch, L., Höck, M., Bertau, M., 2017. Lithium market research – global supply, future demand and price development. Energy Storage Materials 6, 171–179. https://doi.org/10.1016/j.ensm.2016.11.004

Matos, L.P., De Andrade, H.M., Marinato, C.S., De Oliveira Prado, I.G., Coelho, D.G., Montoya, S.G., Kasuya, M.C.M., De Oliveira, J.A., 2020. Limitations to Use of Cassia grandis L. in the Revegetation of the Areas Impacted with Mining Tailings from Fundão Dam. Water Air Soil Pollut 231, 127. https://doi.org/10.1007/s11270-020-04479-0

Melis, A., Spangfort, M., Andersson, B., 1987. Light-absorption and electron-transport balance between Photosystem II and Photosystem I in spinach chloroplasts. Photochem & Photobiology 45, 129–136. https://doi.org/10.1111/j.1751-1097.1987.tb08413.x

Meyer, S.T., Castro, S.R., Fernandes, M.M., Soares, A.C., De Souza Freitas, G.A., Ribeiro, E., 2016. Heavy-metal-contaminated industrial soil: Uptake assessment in native plant species from Brazilian Cerrado. International Journal of Phytoremediation 18, 832–838. https://doi.org/10.1080/15226514.2016.1146224

Montoya, D., Rogers, L., Memmott, J., 2012. Emerging perspectives in the restoration of biodiversity-based ecosystem services. Trends in Ecology & Evolution 27, 666–672. https://doi.org/10.1016/j.tree.2012.07.004

Moreira, T. G., e De Oliveira, A. M. 2016. Dormancy and germination of *Hymenaea stigonocarpa* Mart. Ex Hayne (FABACEAE). REVISTA UNIARAGUAIA, *10* (10), 224-239. (in Portuguese)

Motta, V. H. M., Marques, M. H., Morais, J. F., Alves, W. B., de Oliveira, T. A. F., Adão, B. G. F., de Morais, C. R. 2019. Overcoming dormancy of *Hymenaea courbaril*, using different artificial methods. *Revista GeTeC*, *8* (22). (in Portuguese)

Mukhopadhyay, S., Masto, R.E., Yadav, A., George, J., Ram, L.C., Shukla, S.P., 2016. Soil quality index for evaluation of reclaimed coal mine spoil. Science of The Total Environment 542, 540–550. https://doi.org/10.1016/j.scitotenv.2015.10.035

Naranjo, M.A., Romero, C., Bellés, J.M., Montesinos, C., Vicente, O., Serrano, R., 2003. Lithium treatment induces a hypersensitive-like response in tobacco. Planta 217, 417–424. https://doi.org/10.1007/s00425-003-1017-4

Nardi, S., Pizzeghello, D., Muscolo, A., Vianello, A., 2002. Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry 34, 1527–1536. https://doi.org/10.1016/S0038-0717(02)00174-8

Nascimento, H.H.C.D., Nogueira, R.J.M.C., Silva, E.C.D., Silva, M.A.D., 2011. Jatoba (*Hymenaea courbaril* L.) seedling growth analysis at different water levels in the soil. Rev. Árvore 35, 617–626. https://doi.org/10.1590/S0100-67622011000400005 (in Portuguese)

Nautiyal, P. C., Rachaputi, N. R., Joshi, Y. C. 2002. Moisture-deficit-induced changes in leaf-water content, leaf carbon exchange rate and biomass production in groundnut cultivars differing in specific leaf area. Field crops research, 74(1), 67-79.

Negreiros, D., Fernandes, G.W., Efremova, A.A., Le Stradic, S., Neves, A.C.O., 2016. Growth–survival trade-off in shrub saplings from Neotropical mountain grasslands. South African Journal of Botany 106, 17–22. https://doi.org/10.1016/j.sajb.2016.05.015

Oxborough K., Baker N.R. 1997: Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components: calculation of qP and Fv'/Fm' without measuring F0'. Photosynthesis Research 54: 135-142.

Padilla, F.M., Ortega, R., Sánchez, J., Pugnaire, F.I., 2009. Rethinking species selection for restoration of arid shrublands. Basic and Applied Ecology 10, 640–647. https://doi.org/10.1016/j.baae.2009.03.003

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., et al. 2013. New handbook for standardised measurement of plant functional traits worldwide. Aust. J. Bot. 61, 167. https://doi.org/10.1071/BT12225

Pi, Z., Stevanato, P., Sun, F., Yang, Y., Sun, X., Zhao, H., Geng, G., Yu, L. 2016. Proteomic changes induced by potassium deficiency and potassium substitution by sodium in sugar beet. Journal of Plant Research, 129(3), 527–538.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., Mommer, L. 2011. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist, 193(1), 30–50. https://doi:10.1111/j.1469-8137.2011.03952.x

Pourret, O., Lange, B., Bonhoure, J., Colinet, G., Decrée, S., Mahy, G., Séleck, M., Shutcha, M., Faucon, M.-P., 2016. Assessment of soil metal distribution and environmental impact of mining in Katanga (Democratic Republic of Congo). Applied Geochemistry 64, 43–55. https://doi.org/10.1016/j.apgeochem.2015.07.012

R Core Team 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reich, P. B. 2014. The world-wide "fast-slow" plant economics spectrum: a traits manifesto. Journal of Ecology, 102(2), 275–301. https://doi.org/10.1111/1365-2745.12211

Ribeiro, R.V., Souza, G.M., Oliveira, R.F., Machado, E.C., 2005. Photosynthetic responses of tropical tree species from different successional groups under contrasting irradiance conditions. Rev. bras. Bot. 28, 149–161. https://doi.org/10.1590/S0100-84042005000100012

Rios, C.O., Siqueira-Silva, A.I., Pereira, E.G., 2023. Revegetation of mining-impacted sites with a tropical native grass: Constraints of climate seasonality and trace-element accumulation. Journal of Environmental Management 326, 116655. https://doi.org/10.1016/j.jenvman.2022.116655

Rosado, L.S.S., Modolo, G.S., Guimarães, Z.T.M., Dos Santos, V.A.H.F., Rosado, S.I.P., Ferreira, M.J., 2023. Short-term responses of native tree species to site conditions after mining in the Central Amazon. Ecological Engineering 192, 106992. https://doi.org/10.1016/j.ecoleng.2023.106992

Roy, R. N., Saha, B. 2019. Plants response to heavy metal stress, in: Stress physiology of woody plants. CRC Press, pp. 203-258. https://doi:10.1201/9780429190476-10

Ruan, Y.-L., Patrick, J.W., Shabala, S., Slewinski, T.L., 2013. Uptake and regulation of resource allocation for optimal plant performance and adaptation to stress. Front. Plant Sci. 4. https://doi.org/10.3389/fpls.2013.00455

Salt, D.E., Smith, R.D., Raskin, I., 1998. PHYTOREMEDIATION 26.

Scalon, S.D.P.Q., Mussury, R.M., Wathier, F., Gomes, A.A., Silva, K.A., Pierezan, L., Scalon Filho, H., 2005. Storage, germination seeds and initial growth of *Enterolobium contortisiliquum* (Vell.) Morong. Acta Sci. Biol. Sci. 27, 107–112. https://doi.org/10.4025/actascibiolsci.v27i2.1318

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089

Shahzad, B., Tanveer, M., Hassan, W., Shah, A.N., Anjum, S.A., Cheema, S.A., Ali, I., 2016. Lithium toxicity in plants: Reasons, mechanisms and remediation possibilities – A review. Plant Physiology and Biochemistry 107, 104–115. https://doi.org/10.1016/j.plaphy.2016.05.034

Shakoor, N., Adeel, M., Ahmad, M.A., Zain, M., Waheed, U., Javaid, R.A., Haider, F.U., Azeem, I., Zhou, P., Li, Y., Jilani, G., Xu, M., Rinklebe, J., Rui, Y., 2023b. Reimagining safe lithium applications in the living environment and its impacts on human, animal, and plant system. Environmental Science and Ecotechnology 15, 100252. https://doi.org/10.1016/j.ese.2023.100252

Shakoor, N., Adeel, M., Azeem, I., Ahmad, M.A., Zain, M., Abbas, A., Hussain, M., Jiang, Y., Zhou, P., Li, Y., Xu, M., Rui, Y., 2023a. Interplay of higher plants with lithium pollution: Global trends, meta-analysis, and perspectives. Chemosphere 310, 136663. https://doi.org/10.1016/j.chemosphere.2022.136663

Shi, X., Zhang, X., Chen, G., Chen, Y., Wang, L., Shan, X., 2011. Seedling growth and metal accumulation of selected woody species in copper and lead/zinc mine tailings. Journal of Environmental Sciences 23, 266–274. https://doi.org/10.1016/S1001-0742(10)60402-0

Silva, A. C. F., Silveira, L. P., Nunes, I. G., Souto, J. S. 2012. Superação de dormência de *Enterolobium contortisiliquum* Mor.(Vell.) Morong. Scientia Plena, *8* (4).

Silva, F. C., and DA SILVA, F. C. 2009. Manual of chemical analysis of soils, plants and fertilizers. Brasília, DF: Embrapa Technological Information; Rio de Janeiro: Embrapa Soil. (in Portuguese)

Silva, R.F.D., Antoniolli, Z.I., Grolli, A.L., Scheid, D.L., Bertollo, G.M., Missio, E.L., 2018. Growth and zinc tolerance of *Enterolobium contortisiliquum* Vell. seedlings grown in contaminated soil. Ciênc. Florest. 28, 979–986. https://doi.org/10.5902/1980509833374 (in Portuguese)

Silva, R.F.D., Lupatini, M., Antoniolli, Z.I., Leal, L.T., Moro Junior, C.A., 2011. Behavior of *Peltophorum dubium* (Spreng.) Taub., *Parapiptadenia rigida* (Benth.) Brenan e *Enterolobium contortisiliquum* (Vell.) Morong grown in soil contaminated with copper. Ciênc. Florest. 21, 103–110. https://doi.org/10.5902/198050982752 (in Portuguese)

Tanveer, M., Hasanuzzaman, M., Wang, L., 2019. Lithium in Environment and Potential Targets to Reduce Lithium Toxicity in Plants. J Plant Growth Regul 38, 1574–1586. https://doi.org/10.1007/s00344-019-09957-2

Verbruggen, N., Hermans, C., 2013. Physiological and molecular responses to magnesium nutritional imbalance in plants. Plant Soil 368, 87–99. https://doi.org/10.1007/s11104-013-1589-0

Wakeel, A., Steffens, D., Schubert, S. 2010. Potassium substitution by sodium in sugar beet (*Beta vulgaris*) nutrition on K-fixing soils. Journal of Plant Nutrition and Soil Science, 173 (1), 127-134.

Wang, L., Ji, B., Hu, Y., Liu, R., Sun, W., 2017. A review on in situ phytoremediation of mine tailings. Chemosphere 184, 594–600. https://doi.org/10.1016/j.chemosphere.2017.06.025

White, P. J., Bell, M. J., Djalovic, I., Hinsinger, P., Rengel, Z. 2021. Potassium use efficiency of plants. In Murrell, T. S., Mikkelsen, R. L., Sulewski, G., Norton, R. Thompson, M. L. (Eds.), Improving potassium recommendations for agricultural crops. Springer International Publishing. 119–145.

White, P. J., Karley, A. J. 2010. Potassium. In Hell, R., Mendel, R.-R. (Eds.), Cell biology of metals and nutrients. Springer. 199–224

Wright, I.J., Reich, P.B., Westoby, M., et al. 2004. The worldwide leaf economics spectrum. Nature 428, 821–827. https://doi.org/10.1038/nature02403

Yoon, J., Cao, X., Zhou, Q., Ma, L.Q., 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Science of The Total Environment 368, 456–464. https://doi.org/10.1016/j.scitotenv.2006.01.016

Zanchi, C.S., Silva, A.O., Batista, É.R., Peixoto, D.S., Barbosa, M.V., Dos Santos, J.V., Alvarenga, I.F.S., Silva, B.M., Carneiro, M.A.C., 2022. Pre-cultivation with Herbaceous Plants Assists in the Revegetation Process of Iron Mining Tailings with *Enterolobium contortisiliquum*. Water Air Soil Pollut 233, 231. https://doi.org/10.1007/s11270-022-05696-5

Zhao, J., Wang, W., Zhou, H., Wang, R., Zhang, P., Wang, H., Pan, X., Xu, J., 2017. Manganese Toxicity Inhibited Root Growth by Disrupting Auxin Biosynthesis and Transport in Arabidopsis. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.00272

Supplementary Information

LITHIUM ORE TAILINGS HARM THE VEGETATIVE DEVELOPMENT, PHOTOSYNTHETIC ACTIVITY, AND NUTRITION OF TREE SPECIES

Journal name: Environmental Science and Pollution Research

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Supplementary Tables and Figures

Table S1. Additional information on the species used in the experiment.

Species	**Family**	**Distribution range**	**Reference**
Enterolobium contortisiliquum (Vell.) Morong (TAMBORIL)	Fabaceae-Mimosoidae	Wide geographic distribution	Lorenzi, 2002; Scalon et al., 2005 Brack et al., 2011.
Hymenaea stigonocarpa Mart. ex Hayne (JATOBÁ-DO-CERRADO)	Fabaceae-Caesalpinioideae	Restricted geographic distribution	Lorenzi, 2014.
Hymenaea courbaril L. (JATOBÁ-DA-MATA)	Fabaceae-Caesalpinioideae	Restricted geographic distribution	Cordeiro and Osmar, 2022. Nascimento et al., 2011.
Handroanthus impetiginosus (Mart. ex DC.) Mattos (IPÊ-ROXO)	Bignoniaceae	Wide geographic distribution	Salomão et al., 2016.

References

Lorenzi, H. 2002. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. 4.ed. Nova Odessa: Instituto Plantarum, p.368.

Lorenzi, H. 2014. Árvores brasileiras volume1. Nova Odessa: Instituto Plantarum, p. 384.

Scalon, S.D.P.Q., Mussury, R.M., Wathier, F., Gomes, A.A., Silva, K.A., Pierezan, L., Scalon Filho, H., 2005. Armazenamento, germinação de sementes e crescimento inicial de mudas de *Enterolobium contortisiliquum* (Vell.) Morong. Acta Sci. Biol. Sci. 27, 107–112. https://doi.org/10.4025/actascibiolsci.v27i2.1318

Brack, P and Grings, M. 2011. *Enterolobium contortisiliquum*. In: Coradin, L., Siminski, A., Reis, A., Brazil (Eds.), Espécies nativas da flora brasileira de valor econômico atual ou potencial: plantas para o futuro - Região Sul, Biodiversidade. Ministério do Meio Ambiente, Brasília, DF. Cap. 5, pp. 457-460.

Cordeiro, I. M. C. e Osmar, A. L. 2022. *Hymenaea courbaril* jatobá. In: Lidio C.; Camillo, J.; Ima C. G. V. (Eds.), Espécies Nativas da Flora Brasileira de Valor econômico Atual ou Potencial: Plantas para o futuro - Região Norte, Biodiversidade. Ministério do Meio Ambiente, Brasília, DF. Cap. 5, pp. 1056-1062.

Salomão, P and Camillo, J., M. 2016. *Handroanthus impetiginosus*. In: Roberto F. V.; Camillo, J.; Coradin, L, Brazil (Eds.), Espécies nativas da flora brasileira de valor econômico atual ou potencial: plantas para o futuro - Região Centro-oeste, Biodiversidade. Ministério do Meio Ambiente, Brasília, DF. Cap. 5, pp. 801-812.

Nascimento, H.H.C.D., Nogueira, R.J.M.C., Silva, E.C.D., Silva, M.A.D. 2011. Análise do crescimento de mudas de jatobá (*Hymenaea courbaril* L.) em diferentes níveis de água no solo. Rev. Árvore 35, 617–626. https://doi.org/10.1590/S0100-67622011000400005

Table S2. Results of statistical tests for each response trait and interactions between the corresponding factors, with significance values in bold ($p<0.05$).

Response traits	Interaction between factors	Value of statistical test	P-value
F_v/F_m	SUBSTRATE	F-test = 17.349	**<0.001**
	SP	F-test = 8.260	**<0.001**
NPQ	SP:DBE	F-test = 5.067	**<0.03**
ϕPSII	SP: SUBSTRATE:DBE	F-test = 3.492	**0.02**
ETR	SP: SUBSTRATE:DBE	F-test = 3.492	**0.02**
Chlorophyll *a*	SP: SUBSTRATE	F-test = 3.721	**0.01**
Chlorophyll *b*	SUBSTRATE:DBE	F-test = 15.105	**<0.01**
Chlorophyll *total*	SUBSTRATE:DBE	F-test = 4.863	**<0.05**
LN	SP: SUBSTRATE:DBE	F-test = 2.752	**<0.05**
SD	SP:DBE	F-test = 0.740	**<0.05**
SL	SUBSTRATE	T-test = -2.838	**0.005**
	SP	F-test = 37.372	**<0.01**
RL	SUBSTRATE	T-test = -7.456	**0.001**
	SP	F-test = 4.889	**0.003**
LEI	SP	F-test = 4.805	**<0.001**
LER	SP	F-test = 36.783	**<0.001**
LLS	SP	F-test = 27.259	**<0.001**
RDM	SP: SUBSTRATE	F-test = 9.693	**<0.01**
SDM	SUBSTRATE	T-test = - 2360	**0.02**
	SP	F-test = 39.30	**0.001**
LDM	SP: SUBSTRATE	F-test = 6.843	**<0.01**
TDM	SP: SUBSTRATE	F-test = 14.98	**<0.01**
SLA	SP:SUBSTRATE	F-test = 3.501	**0.01**
ALA	SP:SUBSTRATE	F-test = 4.657	**<0.01**
LAR	SP:SUBSTRATE	F-test = 4.650	**<0.01**
TLA	SUBSTRATE	T-test = -6.934	**0.001**
	SP	F-test = 10.412	**0.001**
RSR	SP:SUBSTRATE	F-test = 6.036	**<0.001**
STI	SP	F-test = 0.3906	>0.05
LTI	SP	F-test = 62.654	**<0.001**
RTI	SP	F-test = 95.707	**<0.001**
TTI	SP	F-test = 84.414	**<0.001**
FTCa	SP	F-test = 3.8893	0.029
FTK	-	-	>0.05
FTMg	SP:SUBSTRATE	F-test = 3.761	**0.032**
FTP	-		>0.05
FTN	SP:SUBSTRATE	F-test = 8.427	**0.001**
FTAl	-	-	>0.05
FTMn	-	-	>0.05
FTLi	-	-	>0.05
[Ca]	SP:SUBSTRATE:TISSUE	F-test = 5.196	**<0.001**
[K]	SP:SUBSTRATE:TISSUE	F-test = 2.964	**0.015**

[Mg]	SP:SUBSTRATE:TISSUE	F-test = 2.309	**0.048**
[P]	SP:SUBSTRATE:TISSUE	F-test = 3.160	**0.01**
[N]	SP:SUBSTRATE:TISSUE	F-test = 4.342	**0.001**
[Al]	SP:SUBSTRATE:TISSUE	F-test = 6.876	**<0.001**
[Mn]	SP:TISSUE	F-test = 9.942	**<0.001**
	SP:SUBSTRATE	F-test =18.558	**<0.001**
[Li]	SP:TISSUE	F-test = 4.018	**0.002**

Fv/Fm: maximum quantum yield of PSII; NPQ: non-photochemical quenching; ϕPSII: effective quantum yield of PSII; ETR: electron transport rate; LN: leaf number; SD: stem diameter; SL: stem length; RL: root length; LEI: leaf expansion interval; LER: leaf expansion rate; LLS: leaf life span; RDM: root dry mass; SDM: stem dry mass; LDM: leaf dry mass; TDM: total dry mass; SLA: specific leaf area; ALA: average leaf area; LAR: leaf area ratio; TLA: total leaf area; RSR: root/shoot ratio; STI: stem tolerance index; LTI: leaf tolerance index; RTI: root tolerance index; TTI: total tolerance index; TF: Translocation factor of nutrients or metals; Nutrient or metal concentrations = [X].

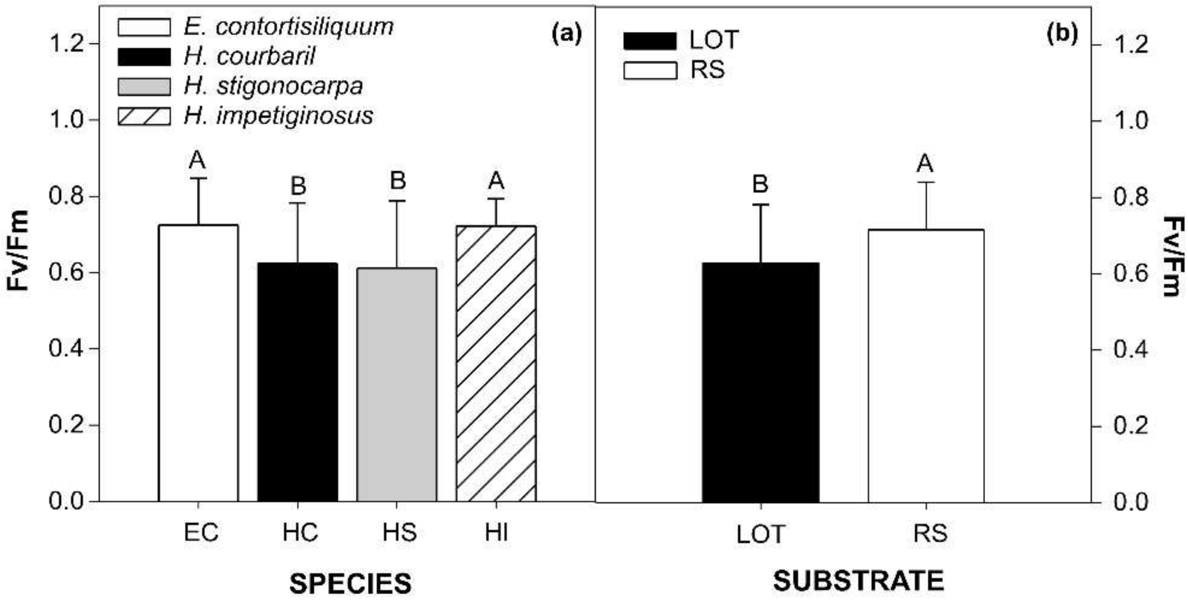

Fig. S1 Maximum quantum yield of PSII (Fv/Fm) measured at 135 DBE and 255 DBE in four woody species grown in Lithium ore tailings (LOT) and Reference Soil (RS) in open chambers. The bars represent the mean per species (a) or the mean of all species (b) with the respective standard deviation. Capital letters compare differences in Fv/Fm between species (a) or between substrates (b, LOT and NS, $p<0.05$).

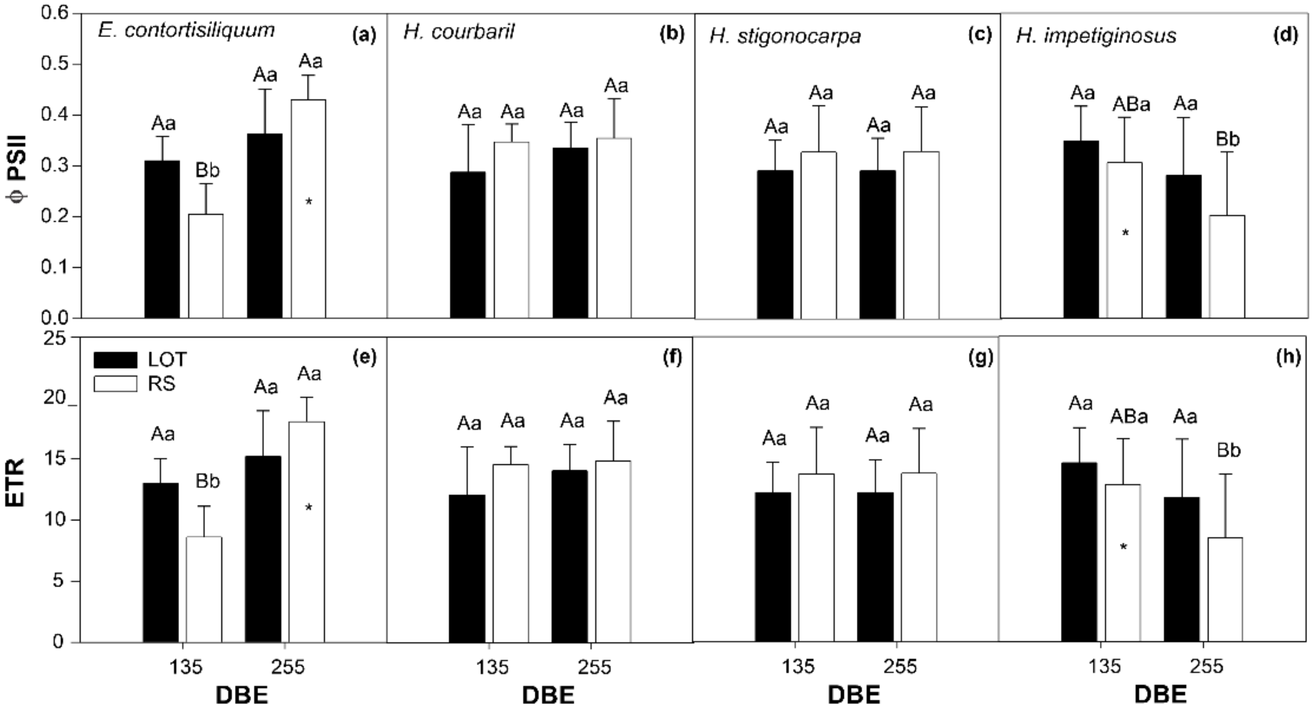

Fig. S2 Photosynthetic variables measured at 135 DBE and 255 DBE in four woody species grown in Lithium ore tailings (LOT) and Reference soil (RS) in open chambers. (a-d) Effective quantum yield (ϕPSII); (e-f) Electron transport rate (ETR). Bars represent means per species and standard deviation for each DBE and substrate. Capital letters compare differences between species in the same substrate and DBE. Lowercase letters indicate differences between substrates in the same DBE for each species. * Indicate differences between DBE in each substrate and species ($p<0.05$).

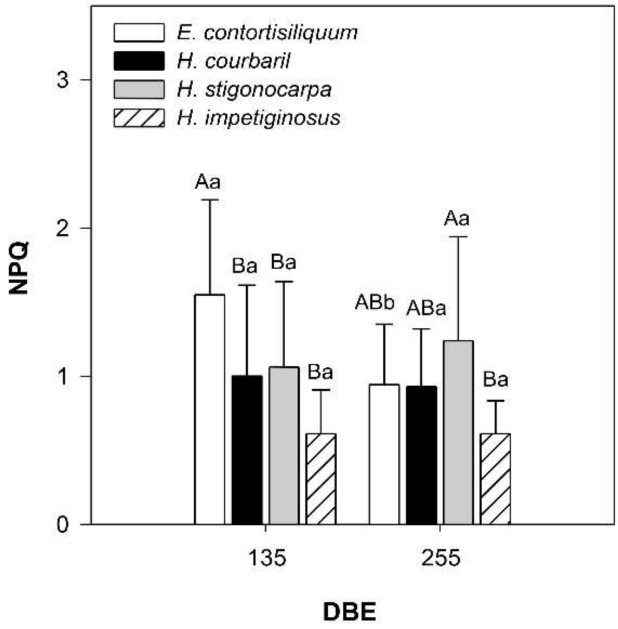

Fig. S3 Non-photochemical quenching (NPQ) measured at 135 DBE and 255 DBE in four woody species grown in Lithium ore tailings (LOT) and Reference soil (RS) in open chambers. Bars represent the mean per species and standard deviation. Capital letters compare NPQ differences between species. Lowercase letters compare differences between DBEs in the same species ($p<0.05$).

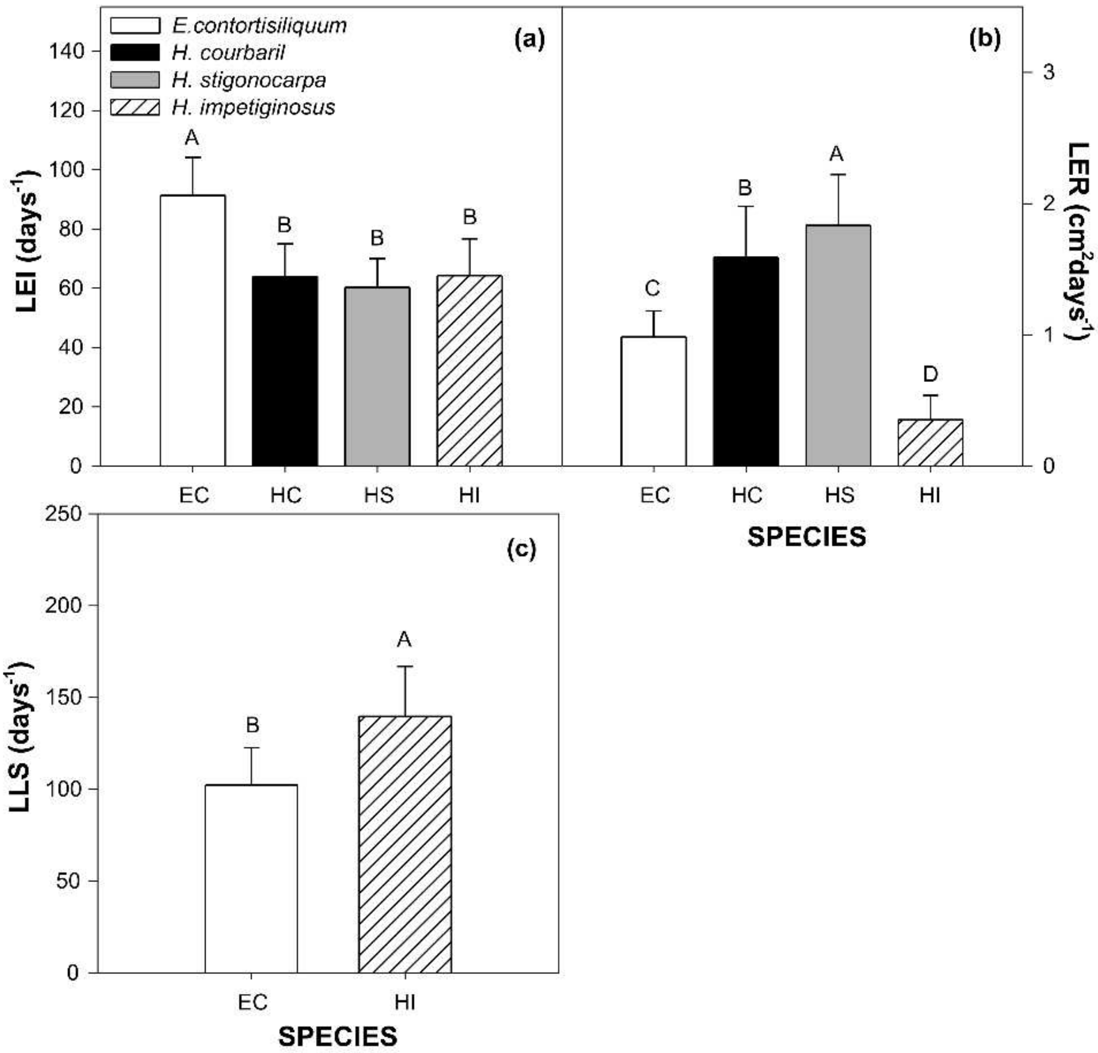

Fig. S4 Leaf development of four woody species grown in Lithium ore tailings (LOT) and Reference soil (RS) in open chambers. (a) LER (cm^2 $days^{-1}$) = leaf expansion rate, (b) LEI (days) = leaf expansion interval, and (c) LLS (days) = leaf life span. The bars represent the averages of all species and the standard deviation. Capital letters compare differences between species ($p < 0.05$).

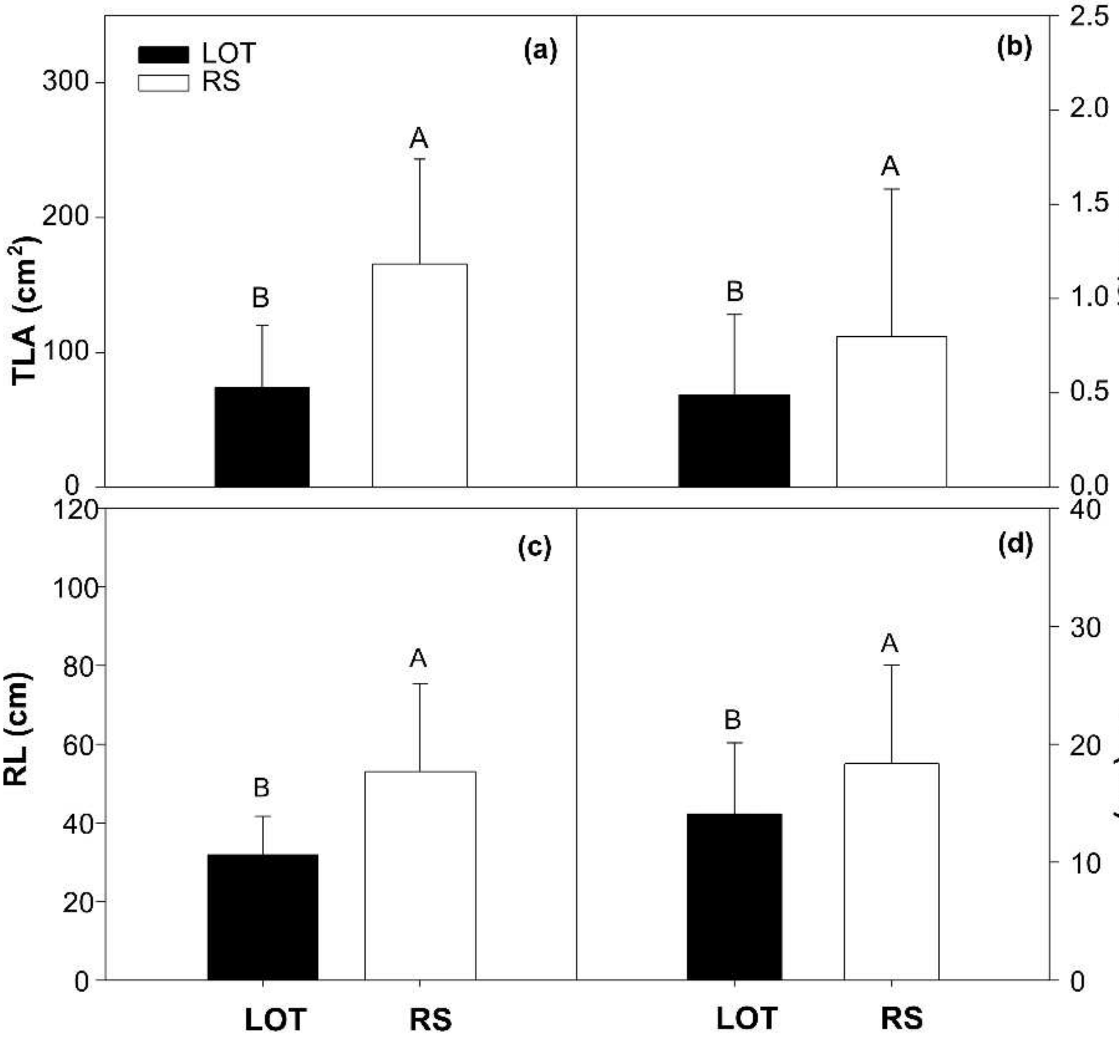

Fig. S5 Morphometric measurements at the end of the experiment of four woody species grown in Lithium ore tailings (LOT) and Reference soil (RS) in open chambers. (a) TLA = total leaf area, (b) SDM = stem dry mass, (c) SL = stem length, (d) RL = length root. Bars represent means and standard deviation. Capital letters compare differences between species or differences between substrates ($p<0.05$).

This article is in the writing refinement phase, is in a non-specific format, and should be submitted to the *Journal of Environmental Management* (Impact factor: 8.7 and Qualis: A1).

CAPÍTULO II

Do intraspecific and interspecific interactions facilitate the initial growth and uptake of metals by *Enterolobium contortisiliquum* (Vell.) Morong in lithium ore tailings?

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Abstract

The growing global demand for lithium, driven mainly by technological advances, has intensified environmental concerns due to the accumulation of contaminating tailings. Understanding how intra- and interspecific interactions influence the growth and tolerance of tree species in lithium ore tailings (LOT) is essential for developing effective recovery strategies. We investigated the early growth of *E. contortisiliquum* across three levels of interaction (individual, intraspecific, and interspecific) in LOT and soil over 280 days. We assessed photosynthetic efficiency, growth, and foliar mineral nutrition. In LOT, interspecific interaction did not favor photosynthetic activity (reduction of Fv/Fm, ϕPSII, and ETR). The absence of an effect of interaction levels on growth and biomass indicates that substrate is the main limiting factor for the development of *E. contortisiliquum*. Interspecific interaction contributed to greater plant vegetative growth only in soil (mainly in leaf production and stem length). Intraspecific interaction intensified competition, impairing growth and biomass (mainly above ground). Competition is the limiting factor for the species' growth in plants growing in the soil. An increase in specific leaf area and leaf area ratio was observed in *E. contortisiliquum* in LOT, indicating an adaptive strategy that optimizes light capture but compromises dry leaf mass. The leaf concentration of nutrients and metals was positively influenced by interspecific interaction in LOT, especially for *E. contortisiliquum*, which had the lowest concentration of metals (Al and Li) and the highest concentration of essential nutrients (K, N, and P) among the species. Both intraspecific and interspecific levels showed a leaf tolerance index > 1, indicating that the presence of other individuals contributed to the tolerance of *E. contortisiliquum*. Interspecific interaction reduced competitive effects, reflected in positive relative interaction index (RII) values, regardless of the substrate. Even without direct gains in biomass or photosynthetic efficiency, interspecific interaction acted as a facilitative mechanism by reducing resource competition and benefiting the stress-sensitive species *E. contortisiliquum*. The results highlight the role of plant interactions in mitigating heavy metal stress and indicate that revegetation strategies based on functional diversity may be more effective than isolated growth plants.

Keywords: Facilitating interactions, Photosynthetic activity, Tolerance Index, Relative Interaction Index, Interspecific interaction.

Introduction

The growing demand for lithium (Li) for industrial production, especially for portable energy storage such as batteries, has raised concerns about the increasing risk of environmental contamination (Aral and Vecchio-Sadus, 2011; Shahzad et al., 2016; Tanveer et al., 2019; Behr et al., 2023; Shakoor et al., 2023a). Lithium mining, and the associated accumulation of mine tailings, has increased markedly in recent decades, following a rise in global lithium production from ~10^4 tonnes in the early 2010s to approximately 2.0–2.4 × 10^5 tonnes in 2023–2024, while mining tailings generated worldwide reach billions of tons per year (Martin et al., 2017; Hudson-Edwards et al., 2024; USGS, 2025). In Brazil, the Li is extracted from spodumene mineral, and the main Li reserve is in the northern Minas Gerais (Braga and França, 2013). The resulting ore tailings after processing spodumene are called aluminum silicate (Al2O34SiO2H2O), and despite Li extraction, approximately 1% remains in the tailings (Castro et al., 2016). Long-term mining tailings deposits constitute an increasing source of environmental contamination affecting ecosystems surrounding contaminated areas (Aral and Vecchio-Sadus, 2011; Shahzad et al., 2016).

Lithium ore tailings (LOT) belong to the silicate group, and in agriculture, silicates are generally added to the soil to correct acidity (Alcarde, 1992; Maio et al., 2011; Freitas et al., 2015). Therefore, a high pH is typical in LOT, and increased pH can lead to plant nutritional deficiency (Aral and Vecchio-Sadus, 2011). Furthermore, the following characteristics are expected in ore tailings: high metal concentrations, fine grain size, and low porosity (Raimondi 2014; Esteves et al., 2020; Zanchi et al., 2022). Fine-grain size and low porosity can contribute to soil compaction (Nardi et al., 2002; Lier et al., 2010), providing a physical barrier to plants' root growth (Esteves et al., 2020). Over time, these substrate characteristics may affect plants' ability to acquire nutrients efficiently (Gagnon et al., 2020; Esteves et al., 2020).

Metals present in tailings can harm plant development (Esteves et al., 2020; Tanveer et al., 2019), and it is considered toxic when they exceed the concentration limits at which plants can develop (Roy et al., 2019; Mahapatra et al., 2020). For example, the effects of Li have already been observed in herbaceous species, cultivars, and/or medicinal plants and recently in tree species (Shahzad et al., 2016; Tanveer et al., 2019; Kastori et al., 2022; Shakoor et al., 2023a; Maciel et al., 2024). It is evident that at high concentrations, Li reduces plant growth, causing oxidative damage to the photosynthetic apparatus and degradation of chlorophyll content, compromising photosynthetic capacity and growth (Tanveer et al., 2019; Li et al., 2009; Maciel et al., 2024). However, even though Li is not considered an essential element for plants, at low concentrations it can stimulate root growth and increase biomass (e.g., Hawrylak-Nowak et al., 2012; Tanveer et al., 2019). Furthermore, Li is alkaline and can compete with the binding sites of essential nutrients such as potassium (K) and sodium (Na), affecting the uptake and translocation of K and Na in plants (Behr et al., 2023). However, the effects of Li concentration on tree species are still poorly understood (Shahzad et al., 2016; Maciel et al., 2024). Therefore, assessing how LOT interferes with the early development of tree species growing alone or competing with other species is crucial for selecting tolerant species with phytoextraction potential to mitigate the environmental impacts of tailings.

Phytoextraction is an environmentally friendly and economically viable technique for rehabilitating mining tailings, using plants capable of removing contaminants to acceptable levels (Salt et al., 1998; Novo et al., 2013). Studies demonstrate that species richness positively affects ecosystem functions and services, such as metal extraction from soils, especially in stressful environments (Kennedy et al., 2002; Maestre et al., 2009; Michalet and Pugnaire, 2016). Facilitative interactions in plant communities are widely recognized (Brooker et al., 2008). Facilitation between plant species and the use of facilitator species have been suggested as an ecological recovery strategy (Brooker et al., 2008). Thus, using different plant species can favor survival, soil stabilization, and ecological succession throughout the rehabilitation process of degraded environments (Nunes et al., 2020).

Facilitation between plant species is especially relevant in degraded environments, where abiotic stress is high (Gómez-Aparicio, 2009; Markham et al., 2011). In mining sites, where soil is nutritionally poor, compacted, or contaminated, facilitating species can improve microenvironmental conditions such as temperature, moisture, nutrient availability, and soil structure (Navarro-Cano et al., 2019; Bashirzadeh et al., 2022). Furthermore, interspecific interactions tend to be more beneficial than intraspecific ones and can reduce physiological stress in young plants and increase their survival, biomass production, and nutrient and metal uptake (Markham et al., 2011; Gómez-Aparicio, 2009; Bashirzadeh et al., 2022). These positive interactions make facilitation an ecologically effective strategy in recovery mined areas (Nemer et al., 2022; Michalet and Pugnaire, 2016).

Enterolobium contortisiliquum (Vell.) Morong (Fabaceae) is a tree widely distributed in Brazil and recommended for revegetation of tailings areas due to its ability to tolerate and accumulate sodium (Na), copper (Cu), zinc (Zn), and aluminum (Al) (Silva et al., 2018; Zanchi et al., 2022). Zanchi et al. (2022) tested the interaction between *E. contortisiliquum* and herbaceous species but observed no significant effects. Our study investigated whether the interaction with tree species could favor their development. Although considered a generalist species and recommended for ecological restoration, *E. contortisiliquum* was not the most tolerant to lithium ore tailings (LOT) in a previous study. However, it showed Al accumulation in its tissues (Maciel et al., 2024). Thus, we evaluated whether intra- or interspecific interactions could improve the growth of *E. contortisiliquum* and increase its potential to revegetate contaminated environments. Three levels of growth interaction were tested: (1) *individual*, with a single individual of *E. contortisiliquum*; (2) *intraspecific interaction*, with eight individuals of *E. contortisiliquum*; and (3) *interspecific interaction*, with eight individuals in total, two from each species: *E. contortisiliquum*, *Hymenaea stigonocarpa*, *H. courbaril, and Handroanthus impetiginosus*. We assume that interaction levels mainly will influence biomass production, photosynthetic activity and metal absorption capacity of *E. contortisiliquum*. We expect, that the species to perform better under interspecific conditions than individual or intraspecific interaction, with: (1) greater growth and biomass production due to improved soil conditions provided by associated species; (2) greater photosynthetic efficiency, reflected in parameters such as chlorophyll *a* fluorescence and chlorophyll content; and (3) greater accumulation of Li in the leaves of the species, indicating phytoextraction potential.

Material and methods

Growth conditions, experimental design, and plant material

The experiment was conducted in plant growth chamber modules at the Laboratory of Functional Plant Ecology (LEFuP) of the Federal University of Viçosa, Florestal Campus (UFV/CAF, 19°52'29" S and 44°25'12" W). The climate in the region is classified as subtropical, with relatively dry winters and rainy summers. We used open chamber modules lined with canvas and wire as modules. The modules have a total volume of 789.12 cm^3 (139 x 96 cm long and 60 cm deep). Each module was subdivided into plots measuring 46.5 × 96 cm long, separated by plywood panels installed from top to bottom to isolate interactions between the plots. Two substrates were used in the experiment: a treatment (lithium ore tailings—LOT) and a control (reference soil—RS). The Soloquímica – Análises para Agricultura e Meio Ambiente laboratory (Brasília, Brazil) performed substrate analyses. Since the same substrates were used in this experiment, the complete results of these analyses, including the table with the physical-chemical data, are available in Maciel et al. (2024).

The selection of the four species in this study was based on a previous study that evaluated the tolerance of tree species to lithium ore tailings (Maciel et al., 2024). The chosen species was *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae), *Handroanthus impetiginosus* (Mart.

ex DC.) Mattos (Bignoniaceae), *Hymenaea stigonocarpa* Mart. ex Hayne (Fabaceae), and *Hymenaea courbaril* L (Fabaceae). *Enterolobium contortisiliquum* was selected as the target species because it is a fast-growing, generalist species, tolerant, and potentially bioaccumulating of Zn, Cu, and Cd (Zanchi et al., 2022). However, despite these characteristics, it demonstrated sensitivity to lithium ore tailings (LOT), even accumulating high concentrations of Al in its tissues (Maciel et al., 2024). All seeds of the selected species were purchased from Arbocenter in May 2022 (seeds collected in the 2020 growing season).

All seeds (except *H. impetiginosus* seeds) received pre-germinative treatment to break physical dormancy (mechanical scarification on the opposite side of the hilum with sandpaper to avoid damage to the hypocotyl-radicle axis). The seeds were sown directly in the plots of each module. Ten modules were used, five in each substrate (five with LOT and five with RS) for each set of species. Each set consisted of three levels of interactions: (1) *individual level*, with a single individual of *E. contortisiliquum*; (2) *intraspecific interaction*, with eight individuals of *E. contortisiliquum*, and (3) *interspecific interaction*, with eight individuals in total, two of each species: *E. contortisiliquum*, *H. stigonocarpa*, *H. impetiginosus*, and *H. coubaril*.

To minimize the influence of environmental factors associated with the physical position of the plots (such as sun exposure time, temperature, and humidity), the order of the interaction levels varied between modules. Within each module, the three plots were organized in different sequences (e.g., starting with the individual level in one module, the intraspecific level in another, and the interspecific level in another) to avoid bias associated with fixed position (Fig. S1). Within each plot, three seeds of each species were sown (in a hole) and irrigated daily throughout the experiment period (November 2022 to August 2023). Only one plant was maintained per hole after the emergence period, when the seedlings were approximately two months old. The sample size per substrate (LOT and RS) consisted of five individual-level plots, five intraspecific-level plots, and five interspecific-level plots.

Vegetative Morphometry

Leaf number (LN), stem diameter (SD) and stem length (SL) were measured weekly for ten months (November 2022 to August 2023, totaling 280 days after the beginning of the experiment, DBE) in *E. contortisiliquum* plants at the three levels of interaction: five plants at the individual level; 20 plants in intraspecific interaction and 10 plants in interspecific interaction, grown in both substrates (LOT and RS). The length and width of two leaves (metaphylls) were monitored weekly from emergence to full growth (leaves with stabilized growth). The leaf stabilization period was defined as the leaf expansion interval (LEI, days). Then, the leaf area was determined after the leaf showed growth stabilization (LA, stabilized width multiplied by stabilized length). The leaf expansion rate was calculated as LA/LEI (LER = LA/LEI, cm^2 $days^{-1}$). The time from leaf emergence on the branch to leaf fall was defined as the leaf life span (LLS, days).

Relative growth rate (RGR) was used to assess plant growth efficiency over time, considering the differences between initial and final sizes among individuals. Relative growth rates were calculated for stem height (HRG), stem diameter (DRG), and leaf number (LRG). The following equation was used to calculate RGR: $RGR = (\ln (W_2) - \ln (W_1)) / (t2 - t1)$. Where: ln represents the natural logarithm; W_1 and W_2 correspond to the initial and final values of the analyzed variable; t1 and t2 indicate, respectively, the start and end times of the evaluated growth interval (month 1 and month 10). The RGR calculation was based on Seki et al., (2012), Daisuke et al., (2013), and Ullah et al., (2023), with appropriate adaptations to the present experiment. The values were calculated individually for each plant and, subsequently, the average was obtained by substrate type (LOT and RS) and by level of interaction (individual, intraspecific, and interspecific, Table S1).

Chlorophyll a fluorescence and chlorophyll indices

Chlorophyll *a* fluorescence and chlorophyll content index were determined at 125 DBE and 275 DBE. In both periods, the number of *E. contortisiliquum* plants analyzed at each interaction level was as follows: individual level, 10 plants (n = 5 on both substrates); intraspecific interaction, 40 plants (n = 20 on both substrates); and interspecific interaction, 20 plants (n = 10 on both substrates).

Chlorophyll *a* fluorescence was measured with a portable fluorometer model PARFluorPen FP110 (Photon System Instruments, Czech Republic), following the NPQ1 protocol. One leaf or leaflet per plant in each substrate was acclimated to the dark with metal clips placed in the central region of the fully expanded leaflets for 30 min. After leaf acclimation, the minimum fluorescence level (F0) was measured during the dark period. Subsequently, a short flash of saturating light (50% intensity = 1,500 µmol $m^{-2\ s-1}$) was applied to reduce the plastoquinone pool and measure the maximum fluorescence in the dark-adapted state (Fm). The values obtained determined Fv/Fm ((Fm − F0)/Fm, (Genty et al., 1989). After a brief relaxation in the dark, the leaf was exposed to actinic irradiance (10% intensity = 100 µmol m^{-2} s^{-1}) for 60 s to stimulate a transient state called the Kautsky effect. Then, during actinic light exposure, five saturating flash pulses (50% intensity = 1,500 µmol m^{-2} s^{-1}) were applied at 12-s intervals to determine NPQ and ϕPSII in a light-adapted state. The electron transport rate (ETR) was calculated using the following formula: ETR = ϕPSII x PAR x 0.5 x la; where 0.5 is the proportion of photons destined for the two photosystems (Melis et al., 1987), la is the leaf absorbance, and PAR is the photosynthetically active radiation.

The chlorophyll content index was determined using an electronic chlorophyll content meter (clorofiLOG, model CFL1030; FALKER, Porto Alegre, BR). Chlorophyll *a*, *b*, and *total* (*a* + *b*) were measured in the same plants and leaves used for chlorophyll *a* fluorescence measurement. Chlorophyll indices were determined optically using the detectable light frequency for chlorophylls. All measurements were performed between 7:00 and 11:00 am.

Concentration of leaf nutrients and metals

Based on a previous study (Maciel et al., 2024), the nutrients and metals with the highest concentration in the LOT were selected to determine their concentrations in the leaves of the species *E. contortisiliquum*, *H. courbaril*, and *H. stignocarpa* in both substrates. The foliar nutritional analysis of *H. impetiginosus* could not be performed due to the absence of replicates of the species in the LOT. The leaf samples were sent in triplicate to the Soloquímica laboratory (Brasília, Brazil) to determine the concentrations of [Ca], [K], [Mg], [P], [N], [Al], [Mn], and [Li]. All samples were dried in an oven for 72 hours at 60 °C until constant weight (He et al., 2015). Subsequently, they were ground in a Wiley mill, sieved with a 1.0 mm mesh (20 mesh), and stored in glass vials for chemical analysis (Carmo et al., 2000). The samples were subjected to wet digestion with HNO_3 and $HClO_4$ (Nitro-Perchloric Digestion, Carmo et al., 2000). From the digested material, it was possible to determine the elements Ca, K, Mg, P, Al, Mn, and Li dissolved in the acid solution and determined in the Agilent MP-AES 4200 Spectrometer (Liberato et al., 2017). The Agilent MP-AES is a compact microwave-induced plasma atomic emission spectrometer based on a highly sensitive nitrogen plasma and an alternative to Flame Atomic Absorption Spectrometry (FAAS), allowing multielemental analysis (Liberato et al., 2017). Nitrogen was determined by the Kjeldahl method and titration after digestion with H_2 SO_4 + H2O2 (Kjeldahl - Sulfuric Digestion, Carmo et al., 2000).

Leaf area, biomass production, and biometric indices

Leaf area was measured at the end of the experiment (280 DBE), considering the following number of *E. contortisiliquum* plants at each interaction level: individual level, 10 plants (n = 5 in each substrate); intraspecific interaction, 80 plants (n = 40 in each substrate); and interspecific interaction, 20 plants (n = 10 in each substrate). Leaf area was measured after removing the petioles. Then, the leaf blades were digitized in black and white using a scanner. Subsequently, the total leaf area (TLA) and the average leaf area (ALA) were calculated using ImageJ 1.x software (Schneider et al., 2012).

The same plants used to measure leaf area were separated into stem and root to determine stem (SL, cm) and root (RL, cm) length, stem dry mass (SDM, g), leaf dry mass (LDM, g), root dry mass (RDM, g), and total dry mass (TDM, g). The leaves, stems, and roots were oven-dried for 72 h at 60 °C (He et al., 2015). The masses were determined on a semi-analytical balance at LEFuP/UFV. The sum of the dry mass of each plant's stem, leaf, and root resulted in TDM. After all measurements, the following biometric indices were calculated: leaf area ratio (LAR, total leaf area/total dry mass, cm^2 g^{-1}), specific leaf area (SLA, leaf area/leaf dry mass, cm^2 g^{-1}), and root/shoot ratio (RSR, root dry mass/shoot dry mass).

Tolerance Index and Relative Interaction Index

The tolerance index (TI) and relative interaction index (RII) were calculated at the end of the experiment (280 DBE), considering the dry mass of the plants grown in the two substrates and for each level of interaction. The TI was calculated in the same plants used for biomass measurements. Based on dry mass measurements, tolerance indices were calculated for stems (stem tolerance index, STI), roots (root tolerance index, RTI), leaves (leaf tolerance index, LTI), and total biomass (total tolerance index, TTI). The tolerance index was based on the equation used by Meyer et al., (2016), proposed by Nautiyal et al., (2002): TI = dry mass (plant organ) in the tailing/dry mass (plant organ) in the soil.

The relative interaction index (RII) was used to quantify the net effects of biotic interactions on species biomass. The index was adapted from the equation used by Wang et al. (2014) and proposed by Armas et al. (2004) as follows: RII = Bw-Bo/Bw-Bo. Bw is the mass observed when *E. contortisiliquum* grew with other plants (inter- or intraspecific interaction) for each substrate. Bo is the expected biomass of *E. contortisiliquum* growing without interaction (individual level), divided by the species richness (S) in the interspecific (S = 3) and intraspecific (S = 1) interaction. The RII represents the ratio between net biomass loss/gain caused by the interaction and the biomass affected only by facilitating and competitive effects.

The values of this index range from -1 to 1, being neutral around zero, negative values for competition, and positive values for facilitation.

Statistical analysis

The mean and standard deviation were calculated for physiological measurements, vegetative morphometry, leaf growth, biomass, biometric indices, tolerance index, relative interaction index, relative growth rate, and leaf nutrient and metal concentrations. Subsequently, the Shapiro-Wilk test was applied to test the dataset's normal distribution. When the dataset did not present normality, we transformed it to base-10 logarithms. Subsequently, the Levene test was applied to verify the homogeneity of variance.

For photosynthetic parameters, a mixed ANOVA with repeated measures was used over time (considering interaction level and substrate as between-subject factors and time as a within-subject factor). A posteriori, Tukey's test was performed to investigate significant differences ($p<0.05$) between interaction level and substrates over time (DBE). For relative growth rate, leaf nutrient and metal concentration, leaf growth, biomass (root, stem, and leaf), and biometric indices, a two-way ANOVA (interaction level and substrate as factors) and Tukey's test were used to compare significant differences ($p<0.05$) between interaction level and substrate. A second two-way ANOVA was also performed for leaf nutrient and metal concentration, considering species and substrate as factors. For the tolerance index, a one-way ANOVA with Tukey's test was performed a posteriori with a significance level of 5% to compare differences between interaction levels. For the relative interaction indices (RII), a permutational ANOVA based on 5,000 permutations was performed, considering a significance level of 5% to compare differences in RII values between interaction levels and between substrates. All statistical analyses and corresponding graphs were performed in R software (version 4.4.1; R Core Team, 2024), using the RStudio environment (version 2023.6.1.524; Posit Team, 2023). The graphs were constructed according to the results of the statistical analysis. The summary of the statistical analysis is available in the supplementary material (Table S2).

Results

Chlorophyll a fluorescence and chlorophyll indices

Independent of the substrate, *E. contortisiliquum* did not show variation in chlorophyll indices between interaction levels, except for total chlorophyll, which showed a higher index when the species was grown alone compared to the intraspecific interaction (p<0,05, Fig. 1, simple effect of interaction level).

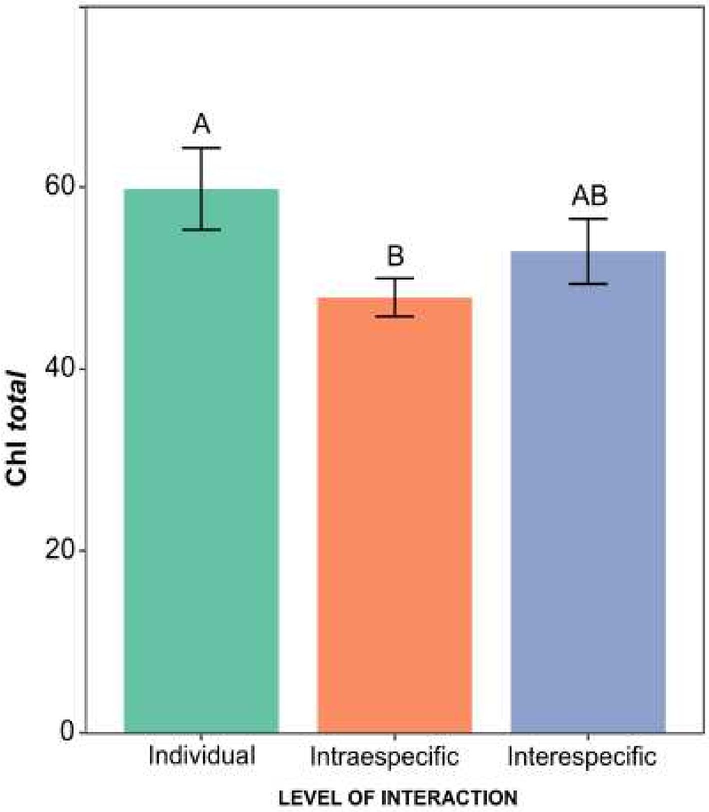

Fig. 1 Total chlorophyll of *E. contortisiliquum* at three levels of interaction (individual, intraspecific, and interspecific) grown in Lithium Ore Tailings (LOT) and reference soil (RS). Bars represent means and standard deviations. Capital letters compare differences between levels of interaction (simple effect, p<0.05).

The Fv/Fm was higher in the plants in RS than in LOT (p<0,05, simple effect of the substrate, Fig. 2a). In both substrates, ETR and ϕPSII were higher at 125 DBE compared to 275 DBE. Despite DBE, ETR and ϕPSII were higher in RS than LOT (p<0,05, Fig. 2b, c, simple effect of DBE and substrate). At 125 DBE, chlorophyll *b* content was higher in RS than in LOT, but this difference was not maintained at 275 DBE. Furthermore, at 275 DBE, chlorophyll *b* content decreased only in RS (p<0,05, Fig. 2d, interaction between substrate and DBE). There were no differences in chlorophyll *a*, chlorophyll *total*, or NPQ values in *E. contortisiliquum* plants in LOT and RS.

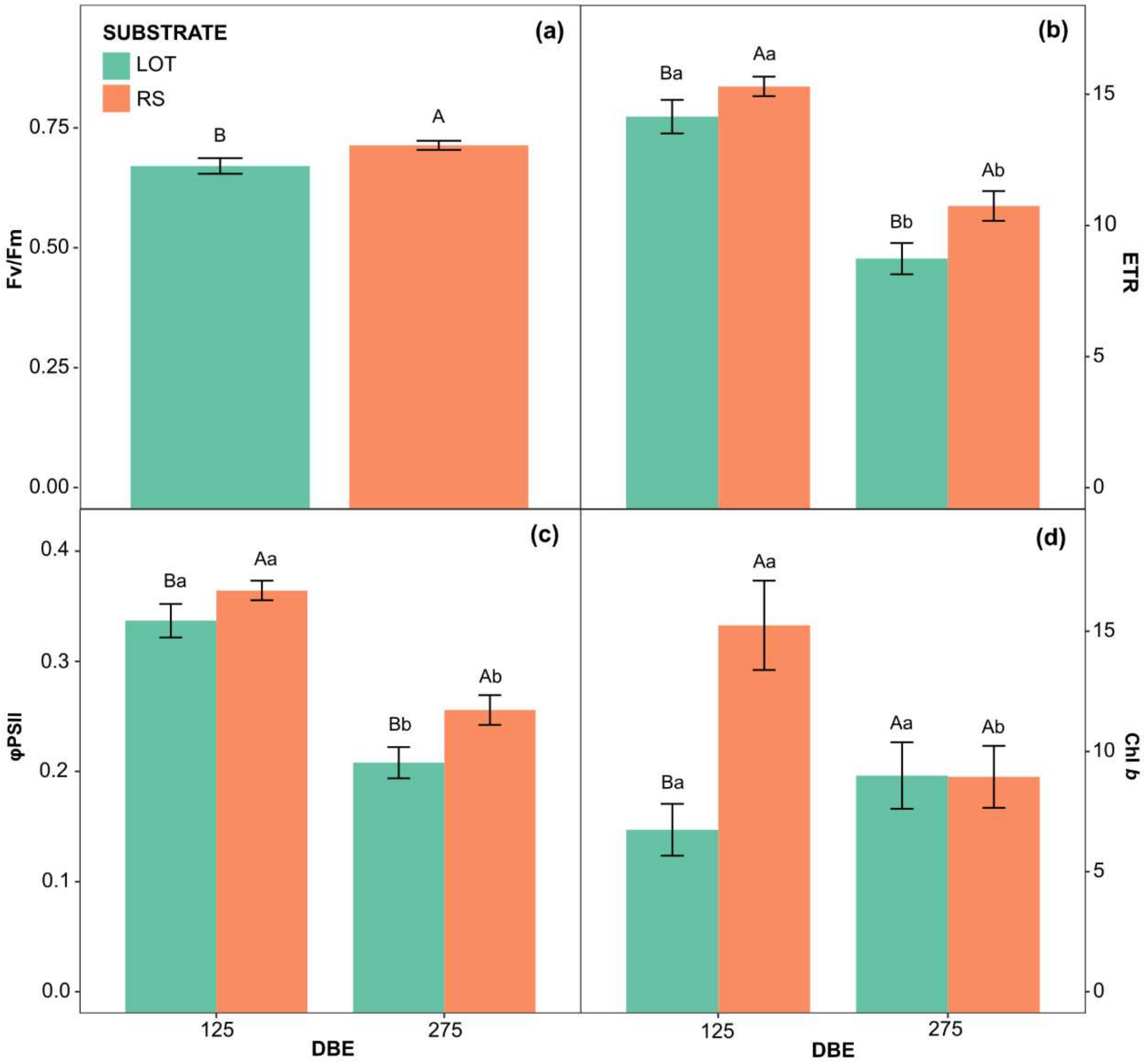

Fig. 2 Photosynthetic parameters of *E. contortisiliquum* at three levels of interaction (individual, intraspecific, and interspecific) grown in Lithium Ore Tailings (LOT) and reference soil (RS). (a) Fv/Fm = maximum quantum yield (single effect of substrate, $p<0.05$); (b) ETR = Electron transport rate; (c) ΦPSII = Effective quantum yield; (d) Chl *b* = Chlorophyll *b*. Bars represent means and standard deviations. Capital letters compare differences between substrates at each DBE. Lowercase letters compare DBE by substrate (double interaction between substrate and DBE, $p<0.05$).

Vegetative morphometry

Leaf production of *E. contortisiliquum* did not differ between substrates. LRG was highest when plants were grown individually (without interaction) and showed the lowest values under intraspecific interaction, regardless of the substrate (Table S1).

Enterolobium contortisiliquum plants showed higher DRG and HRG in RS than in LOT growing alone and under interspecific interaction ($p<0{,}05$, Fig. 3a-b, double effect between substrate and interaction level). There were no differences in DRG and HRG in LOT between the different interaction levels ($p<0{,}05$, Fig. 3a-b, simple effect of substrate). However, in RS, DRG was lower in intraspecific interaction than in other interaction levels (p<0,05, Fig. 3a, double effect between interaction levels and substrate). Also in RS, plants growing alone showed higher HRG, while those in intraspecific interaction showed the lowest values (p<0,05, Fig. 3b, double effect between interaction levels and substrate).

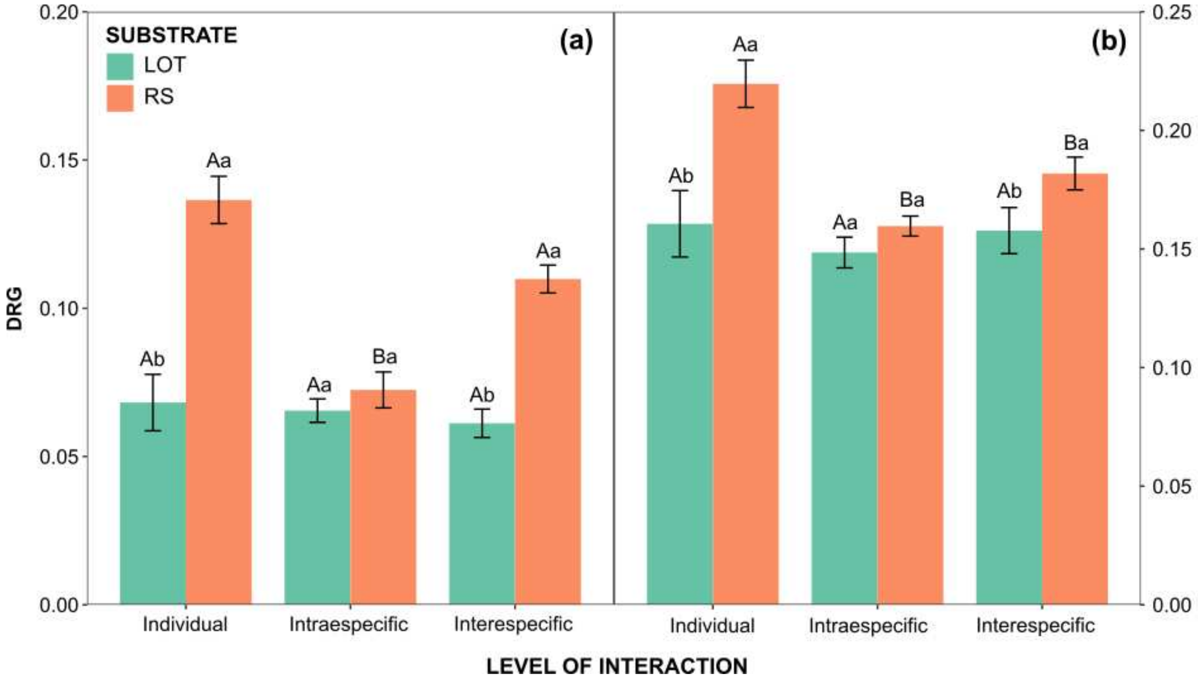

Fig. 3 Stem growth of *E. contortisiliquum* at three levels of interaction (individual, intraspecific, and interspecific) grown in Lithium Ore Tailings (LOT) and reference soil (RS). (a) DRG = relative growth rate of stem diameter; (b) HRG = relative growth rate of stem length in height. Bars represent means and standard deviations. Capital letters compare differences between each level of interaction within the same substrate type. Lowercase letters compare differences between substrates within each level of interaction (double interaction between substrate and interaction level, p<0.05).

Leaf growth parameters (LER and LEI) did not differ between interaction levels or between substrates, except for LLS, which was higher in RS than in LOT (p<0,05, Fig. 4, simple effect of substrate).

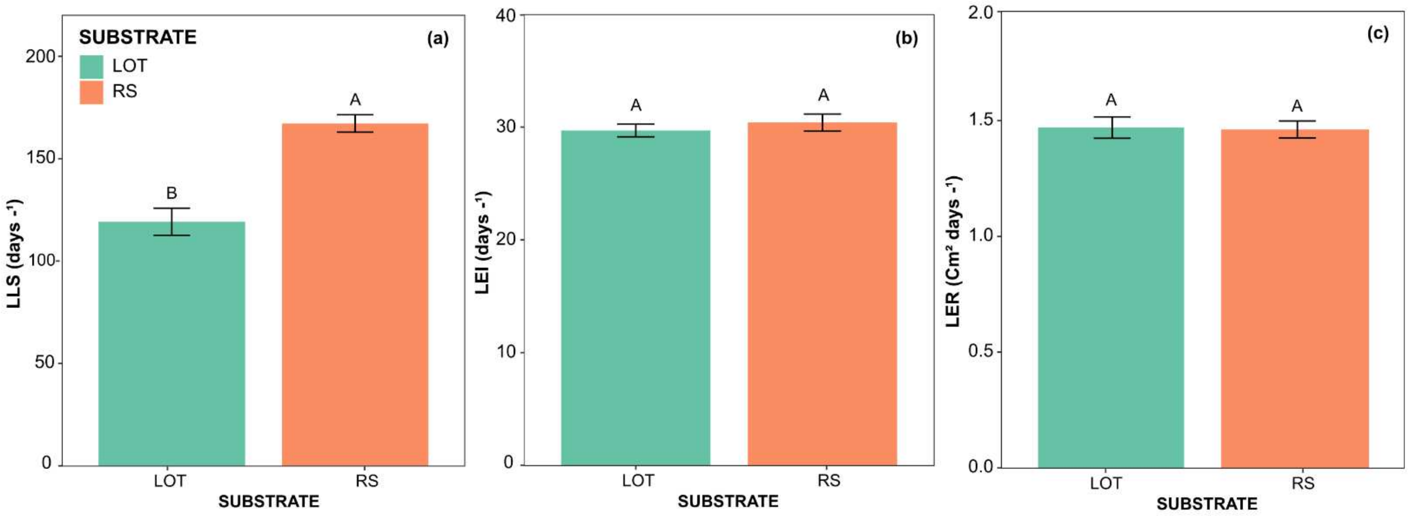

Fig. 4 Leaf development of *E. contortisiliquum* at three levels of interaction (individual, intraspecific and interspecific) grown in Lithium Ore Tailings (LOT) and reference soil (RS). (a) LLS = Leaf Life Span; (b) LEI = Leaf Expansion Interval; (c) LER = Leaf Expansion Rate. Capital letters compare differences between substrates (simple effect of substrate, $p<0.05$).

Leaf nutrient and metal concentrations in E. contortisiliquum and among species

The [Ca], [P], [Mg], and [Li] were higher in the leaves of *E. contortisiliquum* plants in LOT than RS, regardless of the level of interaction ($p<0,05$, Fig. 5a-d, simple effect of substrate). Leaf [N], [K], [Al], and [Mn] did not differ between substrates or between levels of interaction.

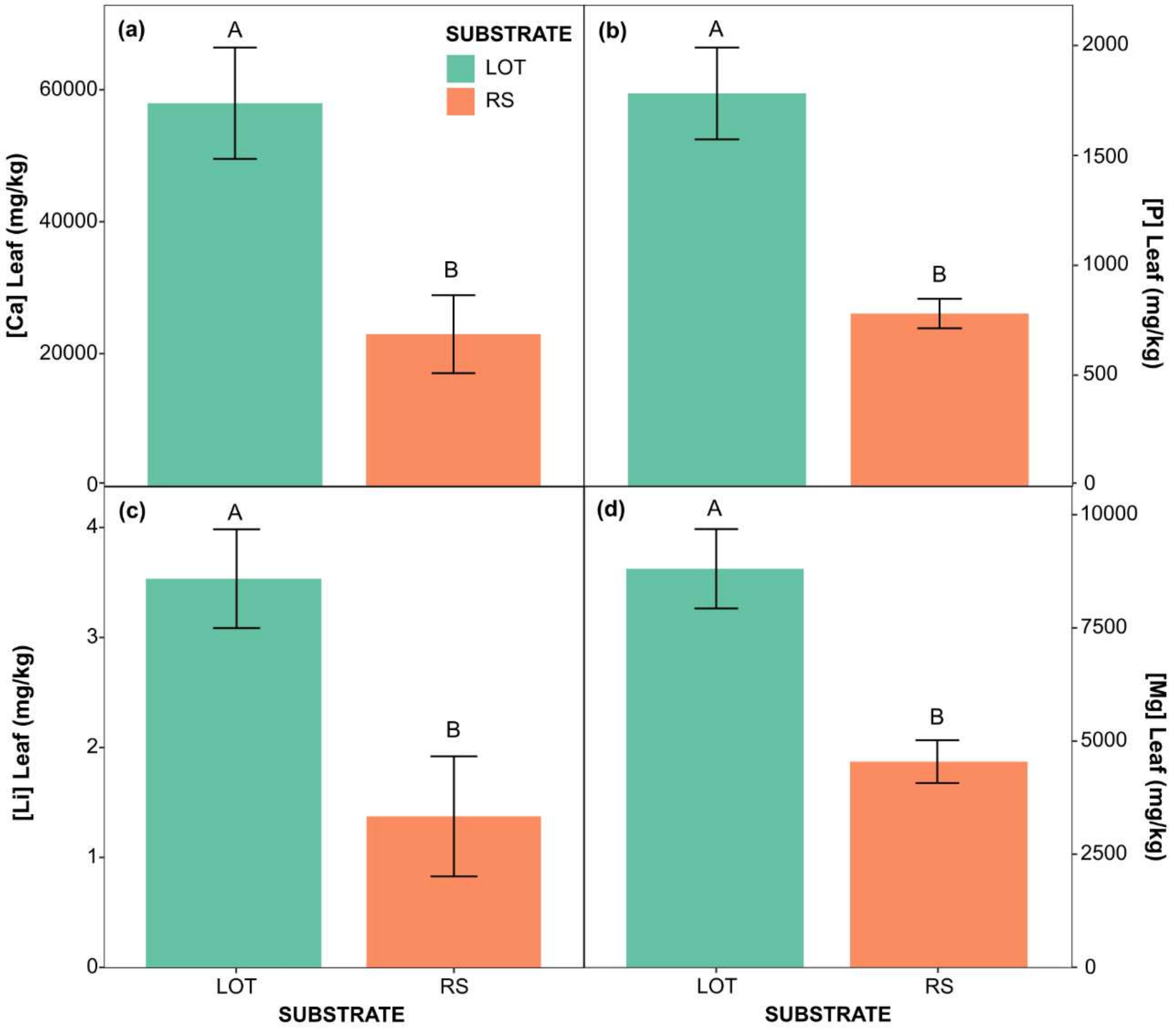

Fig. 5 Concentrations of metals and nutrients (mg/kg) in the leaves of *E. contortisiliquum* at three levels of interaction (individual, intraspecific, and interspecific) grown in Lithium Ore Tailings (LOT) and reference soil (RS). (a) [Ca] = calcium concentrations; (b) [P] = phosphorus concentrations; (c) [Li] = lithium concentrations; and (d) [Mg] = magnesium concentrations. Bars represent means and standard deviations. Capital letters compare differences between substrates (simple effect of substrate, $p<0.05$).

When comparing the leaf concentrations of nutrients and metals between species and substrates, *E. contortisiliquum* presented, in the LOT, the highest [N], [P], [K], and [Mg] among species ($p<0{,}05$, Table 1, interaction between species and substrate). *Hymenaea courbaril* accumulated higher [Li] than *E. contortisiliquum* in the LOT. In RS, *E. contortisiliquum* maintained the highest [K] and [Mg], but showed a reduction in [P] (Table 1, interaction between substrate and species). The three species accumulated higher [Ca] in the LOT but lower [Al] and [Mn]. Furthermore, *E. contortisiliquum* had lower [P] in RS than in LOT, and *H. courbaril* had lower [Li] in RS ($p<0{,}05$, Table 1, interaction between substrate and species).

Table 1. Mean and standard deviation of the concentration of nutrients and metals (mg/kg) in leaf tissue of the species in each substrate (LOT= Lithium ore tailing and RS= Reference soil). Nutrient or metal content is given per 1g dry weight of the sample. All values are means of triplicate ± SD.

Substrate	Species	Concentration of nutrients and heavy metals in leaf tissue							
		N	P	K	Ca	Mg	Al	Li	Mn
LOT	*E. contortisiliquum*	49030.69 ± 13651.05 **Aa**	2026.22 ± 933.58 **Aa**	13173.33 ± 1421.17 **Aa**	47143.88 ± 33560.52 **Aa**	6765.55 ± 776.55 **Aa**	138.11 ± 54.57 **Ab**	2.93 ± 1.09 **Ba**	125.13 ± 27.13 **Ab**
	H. courbaril	21917.07 ± 20201.18 **ABa**	62.66 ± 65.05 **Ba**	4723.33 ± 2498.35 **Ba**	64125.22 ± 13964.36 **Aa**	2148.24 ± 237.96 **Ba**	154.7 ± 59.81 **Ab**	21.13 ± 13.42 **Aa**	220.27 ± 148.3 **Ab**
	H. stigonocarpa	11927.84 ± 4120.6 **Ba**	153.55 ± 92.46 **Ba**	5593.33 ± 1642.14 **Ba**	59811.04 ± 11972.49 **Aa**	1597.23 ± 983.52 **Ba**	161.47 ± 20.59 **Ab**	9.74 ± 5.63 **ABa**	199.64 ± 110.45 **Ab**
RS	*E. contortisiliquum*	37856.76 ± 27194.39 **Aa**	682.27 ± 37.02 **Ab**	11280 ± 1254.55**Aa**	16595.82 ± 9534.33 **Ab**	4512.69 ± 802.89 **Aa**	153.5 ± 6.47 **Aa**	2.15 ± 2.94 **Aa**	269.36 ± 87.63 **Aa**
	H. courbaril	44561.12 ± 7019.72 **ABa**	152.34 ± 175.53 **Aa**	7300 ± 1530.52 **Ba**	4148.96 ± 855.58 **Ab**	299.92 ± 241.61 **Ba**	548.68 ± 285.76 **Aa**	0.7 ± 0.25 **Ab**	849.69 ± 223.81 **Aa**
	H. stigonocarpa	19143.78 ± 12196.82 **Ba**	265.47 ± 243.97 **Aa**	7593.33 ± 1660.55 **Ba**	4699.7 ± 1876.85 **Ab**	223.19 ± 209.59 **Ba**	291.88 ± 83.52 **Aa**	0.53 ± 0.48 **Aa**	733.1 ± 330.75 **Aa**

N: Nitrogen; P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium; Al: Aluminum; Li: Lithium; and Mn: Manganese.

Significant differences and interactions ($p<0.05$): species: substrate.

Capital letters indicate differences between species on the same substrate.

Lowercase letters indicate differences between substrates in the same species.

Biometric indices and biomass production

In *E. contortisiliquum*, SLA and LAR were higher in LOT than in RS (p<0,05, Fig. 6a, c), while ALA, RL, RDM, TDM, and RSR were higher in RS than in LOT (p<0,05, Fig. 6b, d-g, simple effect of substrate). In RS, *E. contortisiliquum* showed higher SDM and LDM at the individual level, but, at the intraspecific level, it exhibited lower SDM, LDM, SL, and LN values (p<0,05, Fig. 7a-d, interaction between substrate and interaction level). In LOT, SDM, LDM, and SL did not differ between interaction levels (p>0,05, Fig. 7a-c). However, LN was lower in the intraspecific interaction compared to the other levels (p<0,05, Fig. 7d, interaction between substrate and interaction level). There was no significant effect in *E. contortisiliquum* at the interspecific level for the morphological variables studied above.

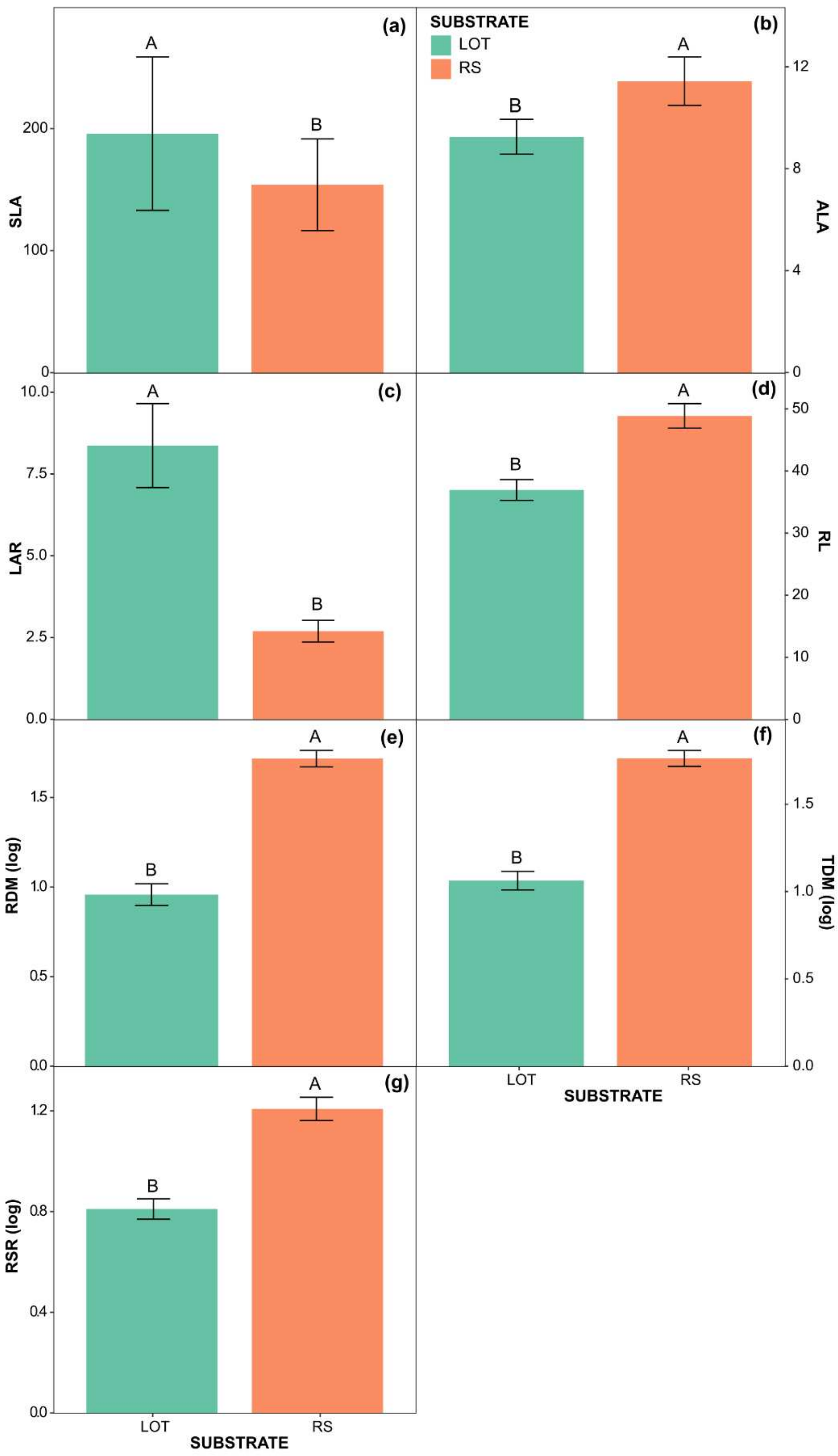

Fig. 6 Morphological characteristics and biometric indices of *E. contortisiliquum* at three levels of interaction (individual, intraspecific, and interspecific) grown in Lithium Ore Tailings (LOT) and reference soil (RS). (a) SLA = Specific leaf area; (b) ALA= Average leaf area; (c) LAR = leaf area ratio; (d) RL = Root length; (e) RDM = Root dry mass; (f) TDM= Total dry mass; (g) RSR = Root to shoot ratio. Bars represent means and standard deviation. Capital letters compare differences between substrates (simple effect of substrate, $p<0.05$).

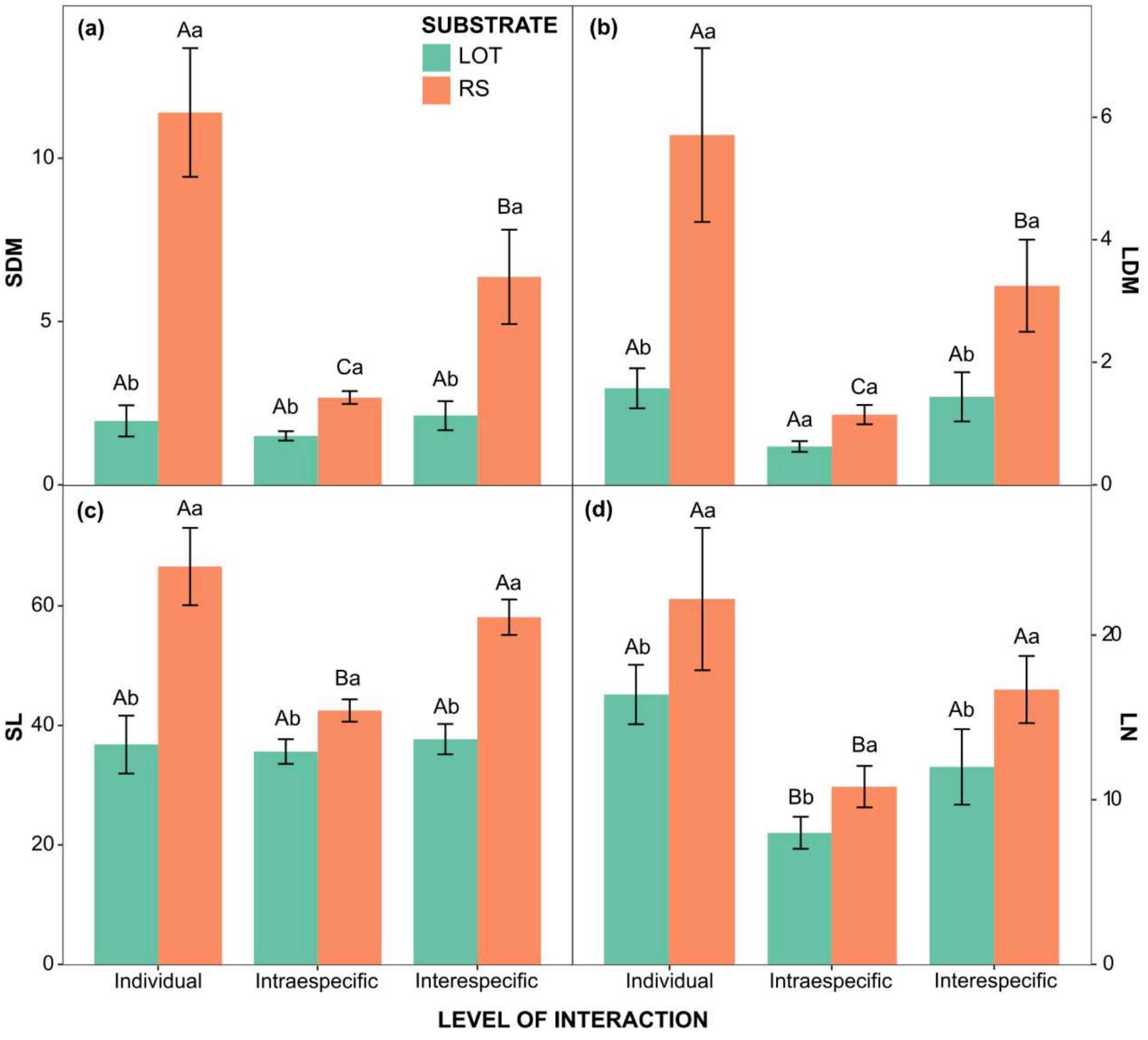

Fig. 7 Morphological characteristics of *E. contortisiliquum* at three levels of interaction (individual, intraspecific, and interspecific) grown in Lithium Ore Tailings (LOT) and reference soil (RS). (a) SDM = Stem dry mass; (b) LDM = Leaf dry mass; (c) SL = Stem length at the end of the experiment; (d) LN = leaf number at the end of the experiment. Bars represent means and standard deviations. Capital letters compare differences between each level of interaction by substrate. Lowercase letters compare differences between substrates within each level of interaction (double interaction between interaction level and substrate, $p<0.05$).

Tolerance Index and Relative Interaction Index

In *E. contortisiliquum*, LTI, RTI, and TTI did not differ between interaction levels (p>0,05, Fig. 8a, c, d). However, the intraspecific and interspecific levels presented LTI > 1 (Fig. 8a). The STI was higher at the intraspecific level compared to the individual level (Fig. 8b). No interaction level reached TTI > 1 (Fig. 8d).

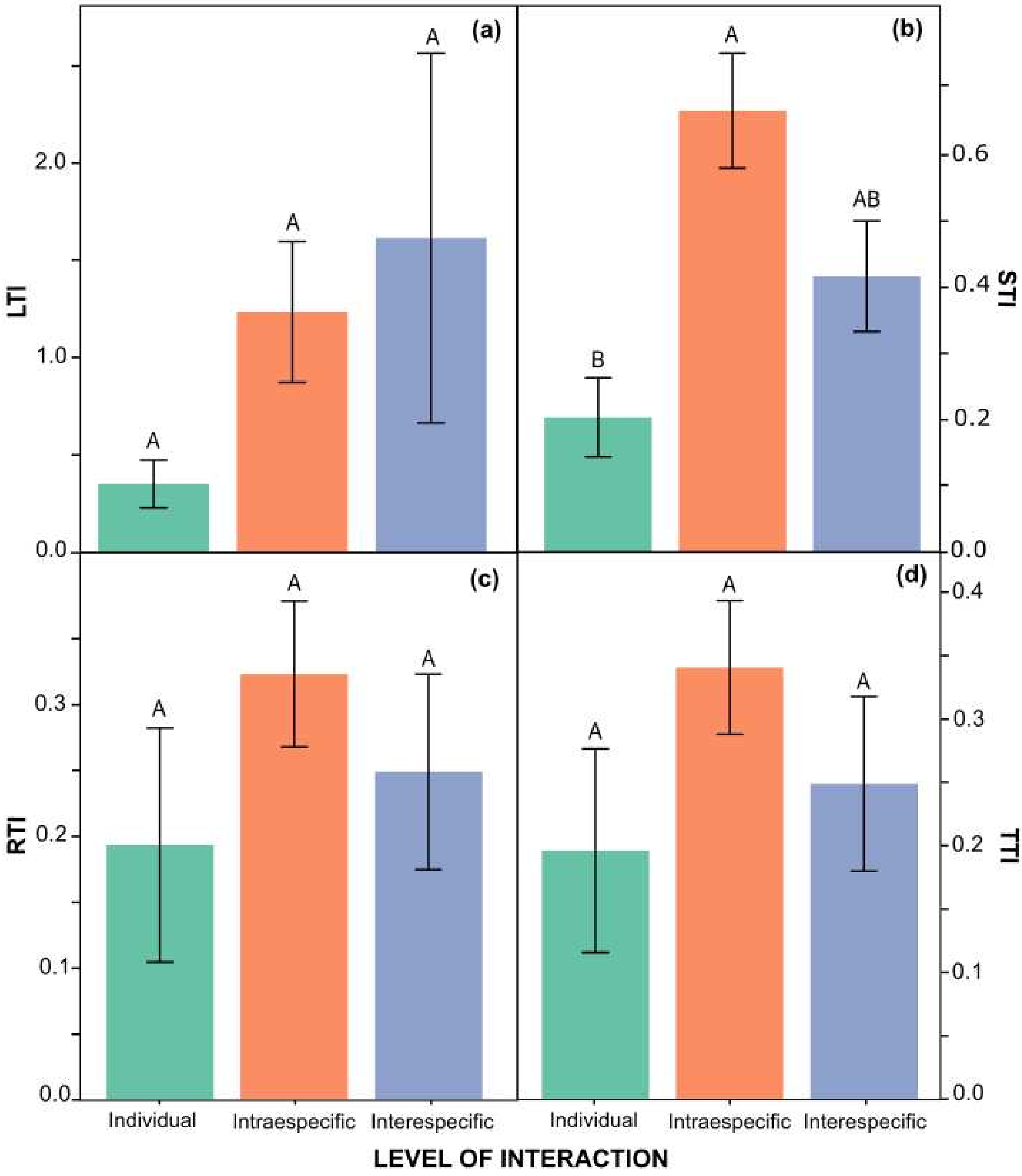

Fig.8 Tolerance index of *E. contortisiliquum* in three levels of interaction (individual, intraspecific, and interspecific) grown in Lithium Ore Tailings (LOT) and reference soil (RS). (a) LTI = leaf tolerance index, (b) STI = stem tolerance index, (c) RTI = root tolerance index, and (d) TTI = total tolerance index (leaf + stem + root). Capital letters compare differences between levels of interaction (simple effect between interaction levels, $p<0.05$).

The intraspecific interaction showed negative RII values, while the interspecific interaction resulted in positive values, regardless of the substrate (Table 2). These results indicate that in both substrates, the intraspecific interaction showed a competitive effect, while the interspecific interaction showed a facilitating effect (Table 2). Furthermore, the type of interaction (intra- or interspecific) significantly influenced RII, regardless of the substrate ($p<0,05$, Table 2).

Table 2. Mean ± standard deviation of the relative interaction index (RII) of *E. contortisiliquum* in three levels of interaction (individual, intraspecific, and interspecific) grown in Lithium Ore Tailings (LOT) and reference soil (RS). Results of permutational ANOVA. ns = not significant; *** p<0.001.

Substrate	Level of interaction	Mean ± ds RII	p-value	Significance
LOT	Interspecific	0.419 ± 0.246		
	Intraspecific	-0.258 ± 0.37		
RS	Interspecific	0.402 ± 0.271		
	Intraspecific	-0.37 ± 0.149		
Effect				
Substrate			0.784	ns
Interaction level			**< 0.001**	***
Substrate: interaction level			0.686	ns

Discussion

Chlorophyll a fluorescence and chlorophyll indices

The reduction in Fv/Fm values in *E. contortisiliquum* grown on lithium ore tailings (LOT) occurred regardless of the interaction levels tested. Similar results were reported in *E. contortisiliquum* plants grown isolated in pots on the same substrates (Maciel et al., 2024), suggesting that substrate quality had a greater influence on photosynthesis than the interaction levels tested (intra- or interspecific). The reduction in Fv/Fm values in plants on LOT indicates a possible impairment of the photochemical efficiency of PSII, potentially limiting the capture or conversion of light energy into chemical energy (Baker and Rosenqvist, 2004; Ribeiro et al., 2005; Maciel et al., 2024).

The decrease in chlorophyll levels reflects a gradual loss of the plants' photosynthetic capacity (Rios et al., 2023). Studies show that lithium concentrations ≥10 mg L^{-1} can reduce chlorophyll content in several plant species by inducing oxidative stress, lipid peroxidation, and structural damage to chloroplasts, resulting in the degradation of photosynthetic pigments (Hawrylak-Nowak et al., 2012; Shakoor et al., 2022). The lithium concentration in LOT was notably higher (262.4 mg dm^{-3}), possibly contributing to the reduction in chlorophyll *b* observed in *E. contortisiliquum* (Maciel et al., 2024). The lower production of chlorophyll *b* may be associated with its greater sensitivity to environmental stress, as it is usually degraded before chlorophyll *a* (Sato et al., 2015). The low efficiency in light energy conversion (Fv/Fm), associated with reducing chlorophyll *b* levels, indicates a possible limitation in PSII repair. This limitation is intensified by the lack of response in increasing NPQ, which compromises the thermal dissipation of excess energy and can permanently damage the photosystems (Allakhverdiev and Murata, 2004).

Our results demonstrate that chlorophyll b content stabilized over time in LOT (Chlorophyll b content was lower in LOT than in RS only at 225 DBE, with no significant difference observed at 275 DBE), which may explain the maintenance of chlorophyll *a* and chlorophyll *total*. Chlorophyll *b* stabilization maintains the integrity of the antenna complex (LHCII), reducing the potential degradation of chlorophyll *a* and consequently maintaining chlorophyll *total* content in this process (Sato et al., 2015). However, over time, the low ϕPSII and ETR values indicate a progressive impairment of photosynthesis, aggravated under heavy metal stress, which affects electron transport, metabolism, and plant growth (Wodala et al., 2012).

Vegetative morphometry

The results indicate that the growth of *E. contortisiliquum* is significantly influenced by the interaction between substrate, exposure period, and level of interaction. At 190 DBE, a hailstorm in the study area caused significant damage to all plants, resulting in widespread defoliation and compromising subsequent leaf production. However, recovery was more evident in plants grown alone, suggesting that competition, especially intraspecific competition, limited leaf production. The absence of variations between the levels of interaction in HGR and DGR in LOT reinforces the role of the substrate as a primary limiting factor (Maciel et al., 2024). In contrast, in RS, the higher DGR and HGR in plants grown alone or in interspecific competition suggest that intra-specific competition becomes the primary modulator of development under less restrictive edaphic conditions. Intraspecific competition resulted in the lowest LN values in the LOT, and DGR and HGR in the RS, reflecting greater ecological overlap and demand for resources, as predicted by classical competition theory (Tilman, 1982; Adler et al., 2018).

Although the substrate did not directly influence leaf production, the longer leaf life span (LLS) observed in RS indicates that the substrate type plays a direct role in leaf longevity. Although LOT has high base saturation, its alkaline pH may have limited the absorption of essential nutrients (Andrade et al., 2018; Zanchi et al., 2022). Furthermore, Mg concentration in leaves exceeded levels considered adequate for plants (>5 g/kg) (Dechen and Nachtigall, 2007; Broadley et al., 2012), which may have compromised metabolic processes and contributed to the reduction in LLS (Maciel et al., 2024). These patterns reinforce that, even in chemically fertile environments, ionic imbalance and substrate alkalinity can cause physiological stress and reduce leaf functional performance, as already reported by Maciel et al. (2024). This can negatively affect photosynthetic capacity and, consequently, the productivity of the species over time.

Leaf nutrient and metal concentrations in E. contortisiliquum and among species

The high availability of Mg, P, and Ca in lithium ore tailings (LOT) is possibly related to the fact that this substrate belongs to the silicate group (Castro et al., 2016). In acidic soils, silicates are commonly applied to correct acidity and make these nutrients available (Alcarde, 1992; Maio et al., 2011; Freitas et al., 2015). This factor may have contributed to the higher Ca, Mg, P, and Li contents observed in the leaves of *E. contortisiliquum* grown in LOT, compared to RS. Plant roots readily absorb Li, with studies indicating significant accumulation in plant tissues, particularly in leaves (Xu et al., 2024). Despite the higher Li content in leaves under LOT cultivation, values remain within the adequate range for more derived plants (angiosperms) (0.2 to 30 mg kg^{-1}; Shakoor et al., 2023b). However, lithium accumulation in leaves is not the expected pattern for *E. contortisiliquum*. According to Maciel et al. (2024), the plant tends to accumulate higher Li concentrations in the stem, with values above 30 mg kg^{-1} in LOT, a toxic level (Shahzad et al., 2016). This accumulation can cause growth restrictions, reduced aboveground biomass production, and decreased leaf area, as observed in C3 herbaceous plants (Shahzad et al., 2016) and *E. contortisiliquum* (Maciel et al., 2024).

The foliar nutritional results, compared with data from Maciel et al. (2024) for the same species grown alone in pots, indicate that growth with other species can favor the reduction of absorption and accumulation of potentially toxic elements, such as Al and Li, in the leaves of *E. contortisiliquum*, *H. courbaril* and *H. stigonocarpa*, as well as Mg in *E. contortisiliquum* and *H. stigonocarpa*. This effect was more evident in *E. contortisiliquum*, where foliar Al contents were reduced in both substrates under growth with other species. These results indicate a possible interspecific facilitation effect in modulating the rhizosphere microenvironment, with a potential impact on the availability or uptake of metals by plants (Wang et al., 2014). These patterns align with recent evidence, including the meta-analysis where Liu et al. (2023a) demonstrated that growth with other species can reduce heavy metal levels in plant tissues and soil. Although *E. contortisiliquum* shares the same functional group as the other legumes evaluated, this species has the potential for nodulation with rhizobia. This characteristic may confer an adaptive advantage in heavy-metal environments (Trannin et al., 2001).

Furthermore, an increase in the concentrations of essential macronutrients, such as Ca, K, and N, was observed in all three species, and in P in *E. contortisiliquum*, indicating that growth with other species generally favored greater foliar nutrient accumulation, regardless of substrate type. This pattern may reflect interspecific facilitation processes or reduced intraspecific competition for resources. Interspecific interactions favor coexistence between species and contribute to mitigating nutritional constraints in contaminated soils, promoting more balanced growth and reduced metal accumulation (Wang et al., 2014; Zeng et al., 2022). Even though nodulation was not observed in this study, previous work indicates that this nodular nitrogen-fixing characteristic is associated with greater absorption of nutrients such as P, K, and Mg (Belane et al., 2014), as observed in *E. contortisiliquum*, which may confer a competitive advantage. Interspecific interaction in more diverse environments can favor the establishment of new species through facilitation, reducing competition for resources and mitigating abiotic stress. This process supports the diversity, productivity and stability of natural plant communities in adverse conditions (Maestre et al., 2009; Michalet and Pugnaire, 2016; Vega-Álvarez et al., 2019; Fagundes et al., 2023).

Although Wang et al. (2014) observed greater metal accumulation in an interspecific cultivation with greater functional diversity, our results point in the opposite direction. In the present study, the interspecific interaction involving *E. contortisiliquum*, *H. courbaril* an *H. stigonocarpa* favored the uptake of essential nutrients and reduced the concentration of heavy metals in the leaves. This divergence may be associated with the low functional diversity among the species used or the smaller number of species in our interspecific experimental treatment.

Biometric indices and biomass production

Specific leaf area (SLA) and leaf area ratio (LAR) were the only biometric indices positively associated with LOT, indicating an adaptive strategy to maximize light capture with less investment in biomass in *E. contortisiliquum* (Liu et al., 2023b). This adjustment, common in stressful environments, results in thinner and lighter leaves, which explains the lower leaf dry mass (LDM) observed in *E. contortisiliquum*. Previous studies indicate that increased SLA, and LAR constitute a functional response associated with both optimized resource acquisition (Liu et al., 2023b) and tolerance to heavy metal toxicity (Hamal et al., 2022; Nescu et al., 2022), reflecting morphological adjustments that allow plants to maintain photosynthesis and functionality under adverse conditions. This morphological pattern observed under LOT is also reflected in the species' physiology, especially its photosynthetic efficiency and biomass accumulation.

In RS, *E. contortisiliquum* showed greater photochemical efficiency, reflected by higher Fv/Fm, ϕPSII, and ETR values, indicating a greater capacity to convert light energy into chemical energy. This photosynthetic efficiency favored greater biomass accumulation, mainly in the shoots (LDM and SDM), compared to LOT. In LOT, the reduction of these parameters suggests functional impairment of the photosynthetic apparatus, limiting growth and biomass production (Wodala et al., 2012; Rios et al., 2023; Maciel et al., 2024). Furthermore, a reduction in LN was observed under intraspecific competition in the LOT. This pattern indicates that the combination of substrate stress and high conspecific density intensifies the adverse effects on development. Both factors simultaneously limit the availability of essential resources, such as water, nutrients, and light, imposing more severe physiological and structural constraints (Grime, 2001; Hamal et al., 2022), compromising the species' performance in degraded environments. Thus, substrate type exerts a predominant influence on *E. contortisiliquum* performance, regulating the effects of intra- and interspecific interactions. While RS favors photosynthetic efficiency and growth, LOT enhances the negative effects of competition, reinforcing the role of substrate as a determining factor in the establishment and development of the species.

Tolerance Index and Relative Interaction Index

Plant interactions, especially in stressful environments such as lithium ore tailings (LOT), can play an important role in plant tolerance (Kennedy et al., 2002; Maestre et al., 2009; Michalet and Pugnaire, 2016). In the case of *E. contortisiliquum*, a leaf tolerance index > 1, both in intraspecific and interspecific interactions, indicates that the presence of other individuals favors the maintenance of functionality even under adverse conditions. Studies show that, in metal-contaminated environments, interspecific interactions can alleviate nutritional or toxic limitations, promote increased biomass, or improve physiological performance for one of the species involved (Martínez-Oró et al., 2017). Although intraspecific competition results in reduced leaf number (LN), the presence of multiple individuals contributes to the partitioning of available metals in the substrate, which can reduce the toxic load absorbed by the plant and minimize adverse effects on the shoot (Nemer et al., 2023). Furthermore, although interspecific interactions do not always promote direct gains in biomass or photosynthetic efficiency, they act as a facilitating mechanism by reducing competition for resources and favoring species that are less tolerant to stress, contributing to a more balanced coexistence in environments with high abiotic pressure (Nemer et al., 2022). These results highlight the positive ecological role of plant interactions in mitigating stress caused by heavy metals. These effects were also observed in the results obtained in the present study, reinforcing the role of interspecific interactions as facilitating strategies under different cultivation conditions.

As expected, interspecific interactions facilitated the greater growth of *E. contortisiliquum* in RS, particularly in relation of LN and SL. In contrast, intraspecific interactions favored resource competition due to ecological overlap (Adler et al., 2018). This scenario, limited vegetative development, leaf production, and biomass production. The growth of *E. contortisiliquum* with other species did not improve its performance in LOT, that is, the interspecific interaction did not compensate for the adverse effects of LOT on photosynthesis and biomass, being considered the primary limiting factor, with competition predominating under extreme stress conditions (Maestre et al., 2009). Under these conditions, intraspecific interactions combined with environmental constraints are even more harmful to *E. contortisiliquum*. However, in less limiting substrates, such as RS, interspecific interactions favored the vegetative growth of *E. contortisiliquum* by reducing competition. Coexistence with functionally distinct species promotes facilitation, increasing the performance and diversity of plant communities (Michalet and Pugnaire, 2016; Fagundes et al., 2023). These results demonstrate that growth with distinct species can offer adaptive advantages by diversifying resource use and creating more stable microhabitats, contributing to the maintenance of biodiversity and the resilience of plant communities in degraded environments.

These patterns indicate that revegetation strategies based on functional diversity may be more effective in environments contaminated by heavy metals. The presence of species with different absorption capacities and tolerances can modulate the rhizosphere microenvironment, reducing the accumulation of toxic metals in tissues, and favor nutritional balance between species. Furthermore, maintaining plant biodiversity contributes to mitigating competition and strengthening ecological processes essential for restoration (Alsherif et al., 2022). Thus, to promote plant growth and increase phytoextraction potential in contaminated areas, it is necessary to adopt diverse plant arrangements that combine stress tolerance with beneficial interspecific interactions.

Implications for ecological restoration and functional revegetation

The results of this study indicate that ecological rehabilitation in lithium mining areas should prioritize functional species arrangements, rather than the isolated introduction of species highly tolerant to soil stress. Tolerant species act as pioneers by establishing themselves under high concentrations of metals, promoting the initial stabilization of the substrate, while coexistence with functionally complementary species reduces the absorption of potentially toxic elements; improves nutritional balance and mitigates the effects of competition (Michalet and Pugnaire, 2016; Vega-Álvarez et al., 2019; Yuan et al., 2022; Fagundes et al., 2023). Therefore, revegetation programs, such as plans for the recovery of degraded areas, should incorporate species consortia with contrasting functional strategies, combining stress tolerance and facilitation, to increase resilience, ecological stability, and successional advancement in mined areas (Maestre et al., 2009; Zeng et al., 2022; Fagundes et al., 2023).

Conclusion

Contrary to expectations, interaction levels did not directly affect photosynthetic efficiency, with LOT substrate quality as the main limiting factor. Our second hypothesis predicted that interspecific interaction would favor the development of *E. contortisiliquum* in LOT. However, this positive effect did not occur in plants in LOT, whereas greater growth happened at the interspecific and isolated levels in RS. The patterns observed in RS indicate that competition is a predominant factor in the development of the species under less restrictive soil conditions. In the case of LOT, the lack of an interaction effect highlights the substrate as the main limiting factor for the development of *E. contortisiliquum*. Interspecific interactions in LOT positively influenced leaf nutrient and metal concentrations, particularly in *E. contortisiliquum*, which showed the lowest metal concentrations and the highest levels of essential nutrients among the species, indicating that species interactions can regulate nutrient and metal uptake under restrictive substrate conditions. In contrast, *H. courbaril* accumulated the highest Li concentrations in its leaves, suggesting a potential phytoextractive role for this species. Leaf tolerance indices were consistently >1 across interaction levels, indicating enhanced species tolerance in the presence of neighboring individuals, while positive RII values across substrates further suggest that interspecific interactions reduced competitive effects. Although interspecific interactions do not always promote direct gains in biomass or photosynthetic efficiency, they act as a facilitation mechanism by reducing competition for resources and favoring species that are more tolerant of stress, contributing to a more balanced coexistence in environments with high abiotic pressure. These results highlight the ecological role of plant interactions in mitigating stress from heavy metals. These patterns indicate that revegetation strategies based on functional diversity may be more effective in environments contaminated by heavy metals. Thus, maintaining plant biodiversity helps mitigate competition and strengthen ecological processes essential to environmental rehabilitation.

References

Adler, P.B., Smull, D., Beard, K.H., Choi, R.T., Furniss, T., Kulmatiski, A., Meiners, J.M., Tredennick, A.T., Veblen, K.E., 2018. Competition and coexistence in plant communities: intraspecific competition is stronger than interspecific competition. Ecology Letters 21, 1319–1329. https://doi.org/10.1111/ele.13098

Alcarde, J. C, 1992. Soil acidity correctors: characteristics and technical interpretations. São Paulo: ANDA, (Technical Bulletin, 6). (in Portuguese)

Alsherif, E.A., Al-Shaikh, T.M., AbdElgawad, H., 2022. Heavy Metal Effects on Biodiversity and Stress Responses of Plants Inhabiting Contaminated Soil in Khulais, Saudi Arabia. Biology 11, 164. https://doi.org/10.3390/biology11020164

Aral, H., Vecchio-Sadus, A., 2011. Lithium: Environmental Pollution and Health Effects, in: Encyclopedia of Environmental Health. Elsevier, pp. 116–125. https://doi.org/10.1016/B978-0-444-63951-6.00531-3

Armas, C., Ordiales, R., Pugnaire, F.I., 2004. Measuring plant interactions: a new comparative index. Ecology 85, 2682–2686. https://doi.org/10.1890/03-0650

Batista, A.Q., Coelho, C.F., Miranda, I.P.D.A., Barbosa, E.M., Miranda, T.D.P.D.A., 2018. The Effect of Different Substrates on the Growth of *Enterolobium contortisiliquum*. JAS 10, 197. https://doi.org/10.5539/jas.v10n3p197

Bebre, I., Marques, I., Annighöfer, P., 2022. Biomass Allocation and Leaf Morphology of Saplings Grown under Various Conditions of Light Availability and Competition Types. Plants 11, 305. https://doi.org/10.3390/plants11030305

Behr, J.H., Zörb, C., 2023. Interactive effect of lithium on concentration of alkali cations in sugar beet (*Beta vulgaris* L.) under saline conditions. J. Plant Nutr. Soil Sci. 186, 38–49. https://doi.org/10.1002/jpln.202200079

Belane, A.K., Pule-Meulenberg, F., Makhubedu, T.I., Dakora, F.D., 2014. Nitrogen fixation and symbiosis-induced accumulation of mineral nutrients by cowpea (Vigna unguiculata L. Walp.). Crop Pasture Sci. 65, 250. https://doi.org/10.1071/CP13283

Braga, P. F. A.; França S. C. A. 2013. Series studies and documents. Lithium: a strategic mineral, 81st ed, IV. CETEM/MCTI, Rio de Janeiro. (in Portuguese)

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B., Michalet, R., 2008. Facilitation in plant communities: the past, the present, and the future. Journal of Ecology 96, 18–34. https://doi.org/10.1111/j.1365-2745.2007.01295.x

Couëdel, A., Alletto, L., Justes, É., 2023. The acquisition of macro- and micronutrients is synergistic in species mixtures: example of mixed crucifer-legume cover crops. Front. Agron. 5, 1223639. https://doi.org/10.3389/fagro.2023.1223639

Daisuke, H., Tanaka, K., Joseph Jawa, K., Ikuo, N., Katsutoshi, S., 2013. Rehabilitation of Degraded Tropical Rainforest Using Dipterocarp Trees in Sarawak, Malaysia. International Journal of Forestry Research 2013, 1–11. https://doi.org/10.1155/2013/683017

Dale, R., Banan, D., Millman, B., Leakey, A.D.B., Mukherji, S., Baxter, I., 2023. Competition for resources during development drives allometric patterns in the grass *Setaria*. https://doi.org/10.1101/2023.12.28.573563

Esteves, G.D.F., De Souza, K.R.D., Bressanin, L.A., Andrade, P.C.C., Veroneze Júnior, V., Dos Reis, P.E., Da Silva, A.B., Mantovani, J.R., Magalhães, P.C., Pasqual, M., De Souza, T.C., 2020. Vermicompost improves maize, millet and *sorghum* growth in iron mine tailings. Journal of Environmental Management 264, 110468. https://doi.org/10.1016/j.jenvman.2020.110468

Fagundes, M.V., Mazzochini, G.G., Ganade, G., 2023. The role of plant diversity and facilitation during tropical dry forest restoration. Journal of Ecology 111, 1231–1241. https://doi.org/10.1111/1365-2745.14091

Faisal, M., Saquib, Q., Alatar, A.A., Al-Khedhairy, A.A. (Eds.), 2020. Cellular and Molecular Phytotoxicity of Heavy Metals, Nanotechnology in the Life Sciences. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-030-45975-8

Feki, K., Tounsi, S., Mrabet, M., Mhadhbi, H., Brini, F., 2021. Recent advances in physiological and molecular mechanisms of heavy metal accumulation in plants. Environ Sci Pollut Res 28, 64967–64986. https://doi.org/10.1007/s11356-021-16805-y

Freitas, L.B.D., Fernandes, D.M., Maia, S.C.M., 2015. Silício na nutrição mineral e acúmulo de alumínio em plantas de arroz de terras altas. Pesqui. Agropecu. Trop. 45, 440–448. https://doi.org/10.1590/1983-40632015v4538085

Gagnon, V., Rodrigue-Morin, M., Migneault, M., Tardif, A., Garneau, L., Lalonde, S., Shipley, B., Greer, C.W., Bellenger, J.-P., Roy, S., 2020. Survival, growth and element translocation by 4 plant species growing on acidogenic gold mine tailings in Québec. Ecological Engineering 151, 105855. https://doi.org/10.1016/j.ecoleng.2020.105855

Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA) - General Subjects 990, 87–92. https://doi.org/10.1016/S0304-4165(89)80016-9

Gómez-Aparicio, L. 2009. The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. Journal of Ecology, 97.6, 1202-1214. https://doi.org/10.1111/j.1365-2745.2009.01573.x

Hamal, J.P., Chettri, M.K., 2022. Impact of heavy metals and biochemical parameters on specific leaf area of roadside trees in Kathmandu, Nepal. EEC 1108–1118. https://doi.org/10.53550/EEC.2022.v28i03.005

Hawrylak-Nowak, B., Kalinowska, M., Szymańska, M., 2012. A Study on Selected Physiological Parameters of Plants Grown Under Lithium Supplementation. Biol Trace Elem Res 149, 425–430. https://doi.org/10.1007/s12011-012-9435-4

Hudson-Edwards, K.A., 2024. Geochemistry and mineralogy of wastes from lithium-bearing granite-pegmatite mining: resource potential and environmental risks. Front. Geochem. 2, 1378996. https://doi.org/10.3389/fgeoc.2024.1378996

Kastori, R., Maksimović, I., Putnikdelić, M., 2022. Lithium in the Environment and its Effects on Higher Plants. Contemporary Agriculture 71, 226–239. https://doi.org/10.2478/contagri-2022-0030

Kennedy, T.A., Naeem, S., Howe, K.M., Knops, J.M.H., Tilman, D., Reich, P., 2002b. Biodiversity as a barrier to ecological invasion. Nature 417, 636–638. https://doi.org/10.1038/nature00776

Li, X., Gao, P., Gjetvaj, B., Westcott, N., Gruber, M.Y., 2009. Analysis of the metabolome and transcriptome of Brassica carinata seedlings after lithium chloride exposure. Plant Science 177, 68–80. https://doi.org/10.1016/j.plantsci.2009.03.013

Liu, Y., Huang, L., Wen, Z., Fu, Y., Liu, Q., Xu, S., Li, Z., Liu, C., Yu, C., Feng, Y., 2023a. Effects of intercropping on safe agricultural production and phytoremediation of heavy metal-contaminated soils. Science of The Total Environment 875, 162700. https://doi.org/10.1016/j.scitotenv.2023.162700

Liu, Z., Zhao, M., Zhang, H., Ren, T., Liu, C., He, N., 2023b. Divergent response and adaptation of specific leaf area to environmental change at different spatio-temporal scales jointly improve plant survival. Global Change Biology 29, 1144–1159. https://doi.org/10.1111/gcb.16518

Maciel, M.G.R., Melo, N.M.J., Oliveira, G.J.S., Baldotto, M.A., Souza, J.P., 2024. Lithium ore tailings harm the vegetative development, photosynthetic activity, and nutrition of tree species. Environ Sci Pollut Res 31, 55187–55203. https://doi.org/10.1007/s11356-024-34707-7

Maestre, F.T., Callaway, R.M., Valladares, F., Lortie, C.J., 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology 97, 199–205. https://doi.org/10.1111/j.1365-2745.2008.01476.x

Mahapatra, K., Banerjee, S., Roy, S. 2020. The hows and whys of heavy metal-mediated phytotoxicity: an insight. Cellular and molecular phytotoxicity of heavy metals, 19-41. https://doi.org/10.1007/978-3-030-45975-8_2

Maio, M.M., Sampaio, R.A., Nascimento, A.L., Prates, F.B.D.S., Rodrigues, M.N., Silva, H.P.D., Dias, A.N., Freitas, C.E.S., 2011. Atributos físicos do solo, adubado com lodo de esgoto e silicato de cálcio e magnésio. Rev. Ceres 58, 823–830. https://doi.org/10.1590/S0034-737X2011000600021

Martin, G., Rentsch, L., Höck, M., Bertau, M., 2017. Lithium market research – global supply, future demand and price development. Energy Storage Materials 6, 171–179. https://doi.org/10.1016/j.ensm.2016.11.004

Martinez-Oró, D., Parraga-Aguado, I., Querejeta, J.I., Conesa, H.M., 2017. Importance of intra- and interspecific plant interactions for the phytomanagement of semiarid mine tailings using the tree species Pinus halepensis. Chemosphere 186, 405–413. https://doi.org/10.1016/j.chemosphere.2017.08.010

Martins Scarpa, A.L., Rodrigues, F.A., Cruz, Y.D.C., Duarte, V.P., De Castro, E.M., Pasqual, M., Pereira, F.J., 2022. Seed germination, initial growth and leaf anatomy of seedlings of four tree species grown in mine tailings in Brazil. Seed Sci. Res. 32, 104–113. https://doi.org/10.1017/S0960258522000174

Meeinkuirt, W., Pokethitiyook, P., Kruatrachue, M., Tanhan, P., Chaiyarat, R., 2012. Phytostabilization of a pb-contaminated mine tailing by various tree species in pot and field trial experiments. International Journal of Phytoremediation 14, 925–938. https://doi.org/10.1080/15226514.2011.636403

Michalet, R., Pugnaire, F.I., 2016. Facilitation in communities: underlying mechanisms, community and ecosystem implications. Functional Ecology 30, 3–9. https://doi.org/10.1111/1365-2435.12602

Nardi, S., Pizzeghello, D., Muscolo, A., Vianello, A., 2002. Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry 34, 1527–1536. https://doi.org/10.1016/S0038-0717(02)00174-8

Nemer, D., Michalet, R., Randé, H., Delerue, F., 2023. The role of ecotypic variation for plant facilitation in a metal-polluted system: Stress-intolerant target ecotypes are the best beneficiaries and stress-tolerant nurse ecotypes the best benefactors. Science of The Total Environment 887, 164134. https://doi.org/10.1016/j.scitotenv.2023.164134

Nemer, D., Michalet, R., Randé, H., Sappin-Didier, V., Delerue, F., 2022. Higher facilitation for stress-intolerant ecotypes along a metal pollution gradient are due to a decrease in performance in absence of neighbours. Oikos 2022, e09499. https://doi.org/10.1111/oik.09499

Nescu, V., Ciulca, S., Sumalan, R.M., Berbecea, A., Velicevici, G., Negrea, P., Gaspar, S., Sumalan, R.L., 2022. Physiological Aspects of Absorption, Translocation, and Accumulation of Heavy Metals in Silphium perfoliatum L. Plants Grown in a Mining-Contaminated Soil. Minerals 12, 334. https://doi.org/10.3390/min12030334

Novo, L.A.B., Covelo, E.F., González, L., 2013a. Phytoremediation of amended copper mine tailings with *Brassica juncea*. International Journal of Mining, Reclamation and Environment 27, 215–226. https://doi.org/10.1080/17480930.2013.779061

POSIT TEAM. RStudio: Integrated Development Environment for R. Version 2023.6.1.524. Boston, MA: Posit Software, PBC, 2023. Available at: https://posit.co/.

R CORE TEAM. R: A language and environment for statistical computing. Version 4.4.1. Vienna, Austria: R Foundation for Statistical Computing, 2024. Available at: https://www.R-project.org/.

Rajkumar, M., Sandhya, S., Prasad, M.N.V., Freitas, H., 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnology Advances 30, 1562–1574. https://doi.org/10.1016/j.biotechadv.2012.04.011

Sato, R., Ito, H., Tanaka, A., 2015. Chlorophyll b degradation by chlorophyll b reductase under high-light conditions. Photosynth Res 126, 249–259. https://doi.org/10.1007/s11120-015-0145-6

Schneider, C. A., Rasband, W. S., Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature methods, 9, 671-675. https://doi.org/10.1038/nmeth.2089

Seki, T., Ohta, S., Fujiwara, T., Nakashizuka, T., 2013. Growth allocation between height and stem diameter in nonsuppressed reproducing Abies mariesii trees. Plant Species Biology 28, 146–155. https://doi.org/10.1111/j.1442-1984.2012.00376.x

Shahzad, B., Mughal, M.N., Tanveer, M., Gupta, D., Abbas, G., 2017. Is lithium biologically an important or toxic element to living organisms? An overview. Environ Sci Pollut Res 24, 103–115. https://doi.org/10.1007/s11356-016-7898-0

Shakoor, N., Adeel, M., Ahmad, M.A., Zain, M., Waheed, U., Javaid, R.A., Haider, F.U., Azeem, I., Zhou, P., Li, Y., Jilani, G., Xu, M., Rinklebe, J., Rui, Y., 2023. Reimagining safe lithium applications in the living environment and its impacts on human, animal, and plant system. Environmental Science and Ecotechnology 15, 100252. https://doi.org/10.1016/j.ese.2023.100252

Silva, R.F.D., Lupatini, M., Antoniolli, Z.I., Leal, L.T., Moro Junior, C.A., 2011. Comportamento de *Peltophorum dubium* (Spreng.) Taub., *Parapiptadenia rigida* (Benth.) Brenan e *Enterolobium contortisiliquum* (Vell.) Morong cultivadas em solo contaminado com cobre. Ciênc. Florest. 21, 103–110. https://doi.org/10.5902/198050982752

Tanveer, M., Hasanuzzaman, M., Wang, L., 2019. Lithium in Environment and Potential Targets to Reduce Lithium Toxicity in Plants. J Plant Growth Regul 38, 1574–1586. https://doi.org/10.1007/s00344-019-09957-2

Trannin, I.C.B., Moreira, F.M.S., Siqueira, J.O., 2001. Growth and nodulation of *Acacia mangium*, *Enterolobium contortisiliquum* and *Sesbania virgata* in heavy metal contaminated soil. https://doi.org/10.1590/S0100-06832001000300024 (in Portuguese)

U.S. Geological Survey. 2025. *Lithium* (Mineral Commodity Summaries 2025). U.S. Department of the Interior. https://pubs.usgs.gov/periodicals/mcs2025/mcs2025-lithium.pdf

Ullah, M., Gulzar, S., Mujtaba, G., 2023. Relative Growth Rate of Tree Seedlings in Mixed Coniferous Forest of Swat, Pakistan. J. Agri. Vet. Sci. 2, 205–211. https://doi.org/10.55627/agrivet.002.03.0463

Vega-Álvarez, J., García-Rodríguez, J.A., Cayuela, L., 2019. Facilitation beyond species richness. Journal of Ecology 107, 722–734. https://doi.org/10.1111/1365-2745.13072

Wang, J., Ge, Y., Chen, T., Bai, Y., Qian, B.Y., Zhang, C.B., 2014. Facilitation Drives the Positive Effects of Plant Richness on Trace Metal Removal in a Biodiversity Experiment. PLoS ONE 9, e93733. https://doi.org/10.1371/journal.pone.0093733

Wang, W., Yang, X., Mo, Q., Li, Y., Meng, D., Li, H., 2023. Intercropping efficiency of Pteris vittata with two legume plants: Impacts of soil arsenic concentrations. Ecotoxicology and Environmental Safety 259, 115004. https://doi.org/10.1016/j.ecoenv.2023.115004

Wodala, B., Eitel, G., Gyula, T.N., Ördög, A., Horváth, F., 2012. Monitoring moderate Cu and Cd toxicity by chlorophyll fluorescence and P_{700} absorbance in pea leaves. Photosynt. 50, 380–386. https://doi.org/10.1007/s11099-012-0045-3

Xu, Z., Peng, S., Pei, L., Zhou, K., Wang, X., 2024. Integrated Analysis of Pollution Characteristic and Ecotoxicological Effect Reveals the Fate of Lithium in Soil-Plant Systems: A Challenge to Global Sustainability. Environ. Sci. Technol. 58, 15755–15765. https://doi.org/10.1021/acs.est.4c02471

Yuan, X., Guo, Z., Duan, C., Yang, J., Tang, H., Li, L., Li, T., Liu, C., 2022. Alleviation of heavy metal stress and enhanced plant complex functional restoration in abandoned Pb–Zn mining areas by the nurse plant Coriaria nepalensis. Front. Ecol. Evol. 10, 1006468. https://doi.org/10.3389/fevo.2022.1006468

Zanchi, C.S., Silva, A.O., Batista, É.R., Peixoto, D.S., Barbosa, M.V., Dos Santos, J.V., Alvarenga, I.F.S., Silva, B.M., Carneiro, M.A.C., 2022. Pre-cultivation with Herbaceous Plants Assists in the Revegetation Process of Iron Mining Tailings with *Enterolobium contortisiliquum*. Water Air Soil Pollut 233, 231. https://doi.org/10.1007/s11270-022-05696-5

Zeng, P., Guo, Z., Xiao, X., Peng, C., Liao, B., Zhou, H., Gu, J., 2022. Facilitation of Morus alba L. intercropped with Sedum alfredii H. and Arundo donax L. on soil contaminated with potentially toxic metals. Chemosphere 290, 133107. https://doi.org/10.1016/j.chemosphere.2021.133107

Zhao, J., Wang, W., Zhou, H., Wang, R., Zhang, P., Wang, H., Pan, X., Xu, J., 2017. Manganese Toxicity Inhibited Root Growth by Disrupting Auxin Biosynthesis and Transport in Arabidopsis. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.00272

Supplementary Information

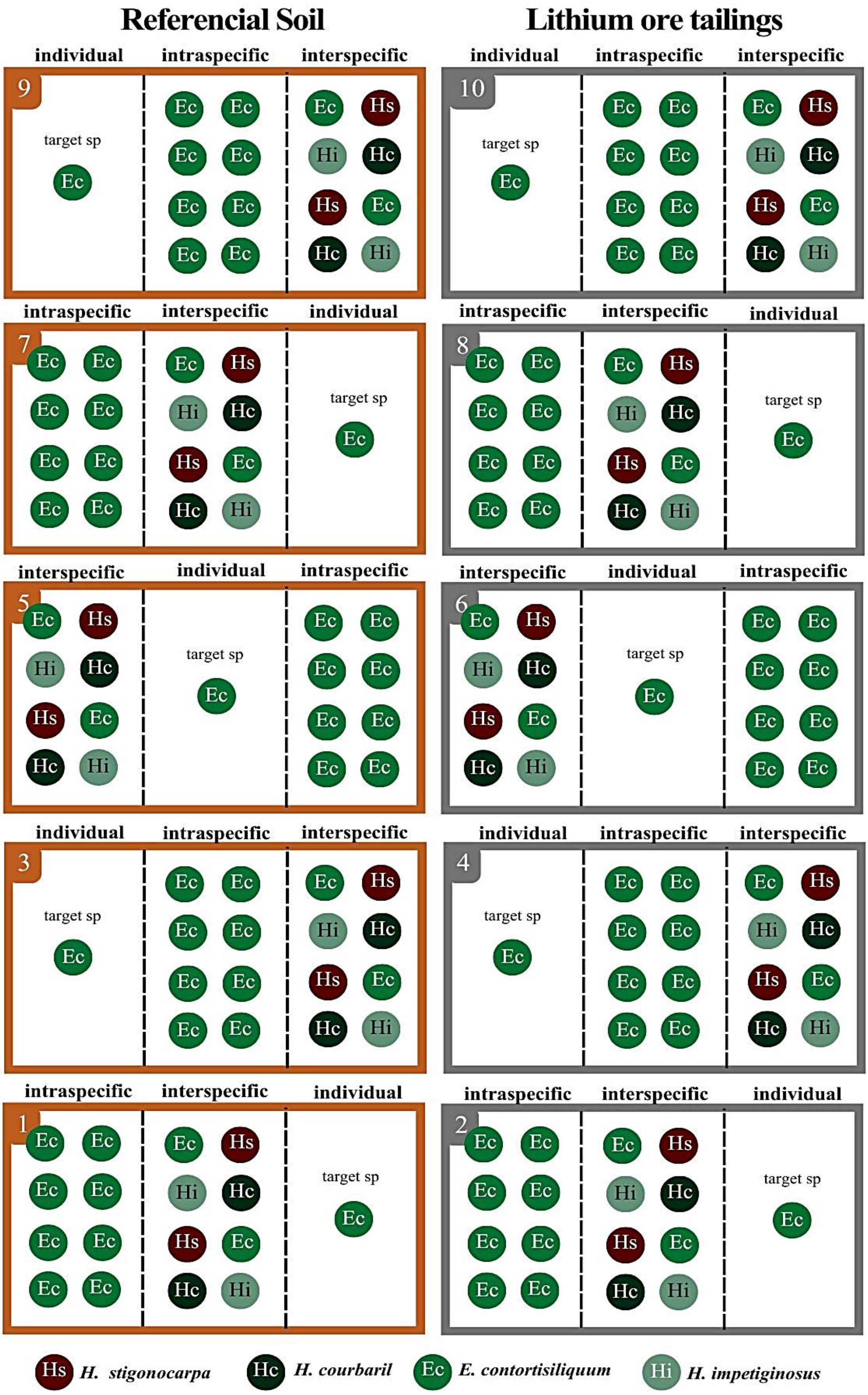

Fig. S1. Schematic representation of the experimental design used to assess individual, intraspecific, and interspecific interactions among woody species under two substrates: referential soil (RS) and lithium ore tailings (LOT). The target species (*Enterolobium contortisiliquum* - Ec) was grown alone (individual), with conspecific individuals (intraspecific), and with heterospecific neighbors (interspecific: *Hymenaea stigonocarpa* - Hs, *Hymenaea courbaril* - Hc, and *Handroanthus impetiginosus* - Hi). Circles indicate plant positions within each experimental unit, with colors and siglas representing species identity. Numbers denote independent replicates. The experiment comprised 10 modules, each containing 3 plots corresponding to the 3 interaction levels, with the spatial arrangement alternating across modules to minimize positional bias. Five modules were established per substrate (RS: modules 1, 3, 5, 7, and 9; LOT: modules 2, 4, 6, 8, and 10), totaling five replicates per interaction level within each substrate.

Table S1. Mean relative growth rate ± standard deviation of stem height (HRG), stem diameter (DRG), and leaf number (LRG) (cm cm/month) of *E. contortisiliquum* at three levels of interaction (individual, intraspecific, and interspecific) grown in lithium ore tailings (LOT) and reference soil (RS).

Substrate	**Level of interaction**	**Growth Rate per month**			**Growth Rate**		
		HRG **(mean ± SD)**	DRG **(mean ± SD)**	LRG **(mean ± SD)**	HRG **(%)**	DRG **(%)**	LRG **(%)**
LOT	Individual	0.161 ± 0.031 **Ab**	0.068 ± 0.021 **Ab**	0.150 ± 0.043 **Aa**	16.1	6.8	15.0
	Intraspecific	0.149 ± 0.029 **Aa**	0.065 ± 0.018 **Aa**	0.055 ± 0.076 **Ab**	14.9	6.5	5.5
	Interspecific	0.158 ± 0.031 **Ab**	0.061 ± 0.015 **Ab**	0.083 ± 0.084 **Aab**	15.8	6.1	8.3
RS	Individual	0.220 ± 0.022 **Aa**	0.137 ± 0.018 **Aa**	0.181 ± 0.042 **Aa**	22.0	13.7	18.1
	Intraspecific	0.160 ± 0.019 **Ba**	0.072 ± 0.027 **Ba**	0.083 ± 0.074 **Ab**	16.0	7.2	8.3
	Interspecific	0.182 ± 0.022 **Ba**	0.110 ± 0.015 **Aa**	0.124 ± 0.069 **Aab**	18.2	11.0	12.4

Significant differences and interactions ($p<0.05$): levels of interaction: substrate.

Capital letters compare differences between each level of interaction within the same substrate type.

Lowercase letters compare differences between substrates within each level of interaction.

Table S2. Results of statistical tests for each response trait and interactions between the corresponding factors. significance values in bold ($p<0.05$) and ns = not significant.

Response traits	**Interaction between factors**	**Value of statistical test**	**P-value**
FACTORS TESTED = SUBSTRATE: INTERACTION LEVEL: DBE			
Fv/Fm	SUBSTRATE	T-test = 2.092	**0.03**
NPQ	-	-	ns
ϕPSII	SUBSTRATE	T-test = -2.255	**0.02**
	DBE	T-test = 8.765	**<0.001**
ETR	SUBSTRATE	T-test = -2.255	**0.02**
	DBE	T-test = 8.765	**<0.001**
Chlorophyll *a*	-	-	ns
Chlorophyll *b*	SUBSTRATE: DBE	F-test = 7.951	**0.047**
Chlorophyll *total*	INTERACTION LEVEL	F-test = 3.255	**0.042**
LN	INTERACTION LEVEL: DBE	F-test = 0.201	**<0.001**

FACTORS TESTED = SUBSTRATO: INTERACTION LEVEL			
DRG	INTERACTION LEVEL: SUBSTRATO	F-test = 12.560	**<0.001**
HRG	INTERACTION LEVEL: SUBSTRATO	F-test = 3.580	**0.03**
LRG	INTERACTION LEVEL	F-test = 7.419	**0.001**
SD	-	-	ns
SL	SUBSTRATE: INTERACTION LEVEL	F-test = 5.340	**0.007**
RL	SUBSTRATE	T -test = -4.777	**<0.001**
LEI	-	-	ns
LER	-	-	ns
LLS	SUBSTRATE	T -test = -6.129	**<0.001**
RDM	SUBSTRATE	T-test = -3.509	**<0.001**
SDM	SUBSTRATE: INTERACTION LEVEL	F-test = 23.247	**<0.001**
LDM	SUBSTRATE: INTERACTION LEVEL	F-test = 10.250	**<0.001**
TDM	SUBSTRATE	T-test = -2.635	**0.009**
SLA	SUBSTRATE	T-test = -2.554	**0.01**
ALA	SUBSTRATE	T-test = 2.754	**0.007**
LAR	SUBSTRATE	T-test = 2.537	**0.01**
TLA	-	-	ns
RSR	SUBSTRATE	T-test = 2.905	**0.004**
FACTORS TESTED = INTERACTION LEVEL			
STI	INTERACTION LEVEL	F-test = 6.330	**0.003**
LTI	-	-	ns
RTI	-	-	ns
TTI	-	-	ns
FACTORS TESTED = SUBSTRATE: INTERACTION LEVEL (in *E. contortisiliquum*)			
[Ca]	SUBSTRATE	T-test = 3.398	**0.004**
[K]	-	-	ns
[Mg]	SUBSTRATE	T-test = 4.275	**0.001**
[P]	SUBSTRATE	T-test = 4.568	**0.001**
[N]	-	-	ns
[Al]	-	-	ns
[Mn]	-	-	ns
[Li]	SUBSTRATE	T-test = 3.059	**0.007**
FACTORS TESTED = SUBSTRATE: SPECIES			
[Ca]	SUBSTRATE	F-test = 5.384	**0.038**
[K]	SPECIES	F-test = 22.053	**<0.001**
[Mg]	SPECIES	F-test = 30.974	**<0.001**
[P]	SUBSTRATE: SPECIES	F-test = 6.416	**0.012**
[N]	SPECIES	F-test = 4.283	**0.039**
[Al]	SUBSTRATE	F-test = 5.377	**0.033**
[Mn]	SUBSTRATE	F-test = 13.881	**0.001**
[Li]	SUBSTRATE: SPECIES	F-test = 3.910	**0.049**

Fv/Fm: maximum quantum yield of PSII; NPQ: non-photochemical quenching; ϕPSII: effective quantum yield of PSII; ETR: electron transport rate; LN: leaf number; RGRD: Relative growth rate Stem diameter;

RGRH: Relative growth rate Stem length; RGRL: Relative Growth Rate number of Leaves; SD: stem diameter; SL: stem length; RL: root length; LEI: leaf expansion interval; LER: leaf expansion rate; LLS: leaf life span; RDM: root dry mass; SDM: stem dry mass; LDM: leaf dry mass; TDM: total dry mass; SLA: specific leaf area; ALA: average leaf area; LAR: leaf area ratio; TLA: total leaf area; RSR: root/shoot ratio; STI: stem tolerance index; LTI: leaf tolerance index; RTI: root tolerance index; TTI: total tolerance index; TF: Translocation factor of nutrients or metals; Nutrient or metal concentrations = [X].

CONSIDERAÇÕES FINAIS

De forma geral, os resultados obtidos nesta tese evidenciaram que o rejeito de minério de lítio (RML) é um fator limitante para o desenvolvimento de espécies arbóreas, afetando o crescimento vegetativo e a eficiência fotossintética. No primeiro capítulo, quando avaliamos o crescimento isolado de diferentes espécies arbóreas em vasos, todas demonstraram ajustes fotossintéticos básicos, mas a permanência do estresse por RML comprometeu a produção de biomassa, a absorção de nutrientes e a tolerância ao longo do tempo. A comparação entre espécies com diferentes distribuições geográficas evidenciou que a tolerância ao RML não é determinada pela amplitude de ocorrência (*H. courbaril* e *H. stigonocarpa* mostraram maior tolerância, enquanto *E. contortisiliquum* foi a espécie mais sensível). Além disso, *H. courbaril* foi a única espécie que demonstrou potencial para fitoextração, ao apresentar tolerância e acúmulo significativo de Li. No segundo capítulo, ao avaliarmos se a interação de *E. contortisiliquum* com outras espécies melhoraria seu desempenho, observamos que a interação interespecífica não compensou os efeitos deletérios do RML sobre a fotossíntese e a biomassa, sendo considerado o fator limitante primário. No entanto, em substrato menos restritivo como o solo comum (SC), a interação interespecífica contribuiu positivamente para o crescimento vegetativo, enquanto a interação intraespecífica intensificou a competição e reduziu a biomassa de plantas jovens de *E. contortisiliquum*. Apesar da ausência de efeito das interações, no RML, observamos que, quando avaliamos a concentração de nutrientes e metais nas espécies em interação interespecífica, *E. contortisiliquum* absorveu maior concentração de nutrientes e reduziu o acúmulo de metais. Esses resultados sugerem que, embora a interação interespecífica não resulte em ganhos diretos de crescimento e fotossintéticos, ela desempenhou um papel ecológico importante ao reduzir parcialmente os efeitos da toxicidade. Além disso, a maior capacidade de algumas espécies de acumular Li (como *H. courbaril*) reforça o papel da diversidade funcional na atenuação dos efeitos tóxicos entre as espécies em interação. De forma geral, os resultados em conjunto destacam a importância de integrar abordagens ecológicas e fisiológicas na revegetação de áreas impactadas por rejeitos de mineração. A seleção de espécies tolerantes, aliada ao cultivo diversificado, pode potencializar a recuperação desses ambientes, ao promover maior estabilidade ecológica e funcional, conciliando a mitigação da toxicidade, a manutenção da biodiversidade e o fortalecimento de processos ecológicos.

ANEXOS

Anexo 1: Artigos da tese publicados – Capítulo I

Maciel, M.G.R., Melo, N.M.J., Oliveira, G.J.S., Baldotto, M.A., Souza, J.P., 2024. Lithium ore tailings harm the vegetative development, photosynthetic activity, and nutrition of tree species. Environ Sci Pollut Res 31, 55187–55203. https://doi.org/10.1007/s11356-024-34707-7

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RESEARCH ARTICLE

Lithium ore tailings harm the vegetative development, photosynthetic activity, and nutrition of tree species

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Abstract

Lithium (Li) exploitation promotes socioeconomic advances but may result in harmful environmental impacts. Thus, species selection for recovering environments degraded by Li mining is essential. We investigated the tolerance and early growth of four tree species to Li ore tailings (LOT), *Enterolobium contortisiliquum* and *Handroanthus impetiginosus* with wide geographic distribution and *Hymenaea courbaril* and *H. stigonocarpa* with restricted geographic distribution. The plants grew in LOT and soil for 255 days to evaluate photosynthesis, growth, and mineral nutrition. LOT negatively affected species growth, reducing the length of stems, roots, and biomass through structural and nutritional impoverishment. LOT favored the accumulation of Mg and decreased the absorption of K. The species presented a reduction in potential quantum efficiency and the chlorophyll index (*b* and *total*). *E. contortisiliquum* was the least tolerant species to LOT, and *H. courbaril* and *H. stigonocarpa* maintained their mass production in LOT, indicating greater tolerance to tailings. Furthermore, *H. courbaril* presented a translocation factor > 1 for Li and Mn, indicating the potential for phytoextraction of these metals. Our results offer first-time insights into the impacts of LOT on the early development of tree species with different geographic distribution ranges. This study may help in the tree species selection with a phytoremediation role, aiming at the recovery of areas affected by Li's mining activity.

Keywords Functional traits · Lithium ore mining · Metals · Nutrient absorption · Chlorophyll · Photosynthesis · Biomass · Tolerance indices

Introduction

Lithium (Li) is a metal widely used worldwide, and its global exploitation has increased in recent years, mainly due to its application in technological items such as batteries (Zhao et al. 2017; Martin et al. 2017). The Li extraction and subsequent processing into Li carbonate and hydroxide generate tailings and chemical residues (Braga and França, 2013). The resulting ore tailings are named aluminum silicate, a subproduct of Li extraction (Castro et al. 2016). Although it contains some essential nutrients such as phosphorus, potassium, calcium, and magnesium and other metals such as manganese and sodium in its chemical composition, the waste still retains approximately 1% of Li after the extraction processes (Castro et al. 2016).

Although different nutrients are essential for plant growth, their toxicity depends on their role in plant development and may vary according to their ambient concentration (Roy et al. 2019). Metals could affect morphophysiological traits in a positive or negative way, and this response is plant-dependent (Anjum et al. 2016; Esteves et al. 2020; Rosado et al. 2023). Tree species with narrow geographical distribution may present leaf necrosis and reduced dry mass production in soils contaminated with high cadmium and zinc concentrations. On the other hand, species with wide

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Anexo 2: Resumo gráfico do Capítulo I