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Adaptive Significance of Nickel Hyperaccumulation by Plants

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ABSTRACT

Nickel (Ni) hyperaccumulation in plants is an unusual attribute that imposes significant physiological costs. Persistence of Ni hyperaccumulation across a range of taxa suggests a significant evolutionary advantage to this trait. This review examines seven non-mutually exclusive hypotheses to explain the evolution of Ni hyperaccumulation in plants. The hypotheses include elemental defense, elemental allelopathy, drought tolerance, inadvertent uptake, enhanced reproductive fitness, incremental advantage, and metal tolerance and disposal. While our main focus is on Ni hyperaccumulation, we also include selected information about the hyperaccumulation of other metals where relevant to the seven hypotheses. Additionally, we discuss common experimental techniques used when studying Ni hyperaccumulation and recognize recent advances in technology available to study hyperaccumulation. We also identify current gaps in research that should be prioritized to help us better understand the evolutionary significance of metal hyperaccumulation.

1 | Background

Hyperaccumulation of metals/metalloids is a relatively rare trait found primarily among plants growing on metalliferous soils (Manara et al. 2020). Historically, it has been defined as the ability to accumulate a metal(loid) to at least 0.1% of a plant's dry weight (van der Ent et al. 2012). However, this definition has been the subject of debate in recent years, as researchers work toward a standardized understanding of what qualifies as a hyperaccumulator. Goolsby and Mason (2015), for instance, proposed that hyperaccumulators be defined by their ability to accumulate leaf metal concentrations above element-specific thresholds when growing in natural soils with adequate metal availability. This definition hinges on recognizing tolerance and hyperaccumulation as continuous, rather than binary, traits. To be considered hyperaccumulators, taxa must not only take up high levels of metals but also tolerate these concentrations well enough to maintain viable populations on metal-rich soils

such as ultramafic, calamine, seleniferous, or other contaminated soils.

Several metals have been observed at extremely high concentrations in plant tissues, with nickel (Ni) being the most frequently hyperaccumulated metal (van der Pas and Ingle 2019). This is likely due to the high frequency at which Ni-rich ultramafic (e.g., serpentine) soils occur in the world (Hulshof and Spasojevic 2020; Garnica-Díaz et al. 2023), along with the use of dimethylglyoxime as a method for easily screening plants for Ni hyperaccumulation in the field (Purwadi et al. 2021; Disinger et al. 2024). Additionally, handheld x-ray fluorescence spectrometers now provide a non-destructive and effective approach for screening herbarium specimens for metal hyperaccumulation, leading to the recent discovery of numerous Ni-hyperaccumulating species from serpentine soils, along with species that also hyperaccumulate cobalt (Co), manganese (Mn), and zinc (Zn) (Gei et al. 2018; van der Ent et al. 2019; Belloeil et al. 2021; Disinger

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et al. 2024). Serpentine soils are often regarded as a model system and, consequently, have received disproportionate attention from botanists relative to other metal-rich substrates. This research bias may partly account for the greater number of reported cases of Ni hyperaccumulation, though this explanation remains unverified. Additionally, Ni, being a divalent cation of similar size to zinc (Zn^{2+}) and iron (Fe^{2+}), can easily utilize existing transporters (Ferrero et al. 2020). While hyperaccumulation of other elements—such as aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), the rare-earth elements (REE), selenium (Se), thallium (Tl), and zinc (Zn)—has also been reported (van der Ent et al. 2012; Reeves et al. 2018), the number of known hyperaccumulator species for most of these elements is substantially smaller than for Ni (Pollard et al. 2014). Notable exceptions include Al hyperaccumulation, which may occur in 25% of all angiosperms depending on the definition applied (Pollard 2023), and Zn/Cd hyperaccumulation, which has been intensively studied in model species such as *Noccaea caerulescens* and *Arabidopsis halleri* (Krämer 2010; Wang et al. 2022). For several other elements, documented cases remain relatively few and the literature comparatively limited, despite geographically widespread occurrences.

In rare cases, multi-element hyperaccumulation has been documented. For example, *N. caerulescens* (Brassicaceae) is known to hyperaccumulate Zn, Ni, and Cd (Wang et al. 2022). Notably, variation in Ni and Cd hyperaccumulation occurs among ecotypes, genetically differentiated populations of the same species that differ in both trait expression and allele frequencies (Stronen et al. 2022). *Noccaea caerulescens* was found to hyperaccumulate Ni when growing on serpentine soils, and several populations also exhibit Cd hyperaccumulation. It is unclear whether the variation in the ability to hyperaccumulate Cd reflects genetic differences or variation in soil Cd exposure (Reeves et al. 2006). However, hyperaccumulation of Ni and Cd appears to be independent of Zn uptake, which remains consistently high regardless of soil chemistry (Lombi et al. 2001; Reeves et al. 2006). In other species, such as *Pteris vittata* (Pteridaceae) and *Viola principis* (Violaceae), co-accumulation of elements appears to be more strongly linked (Wan et al. 2017). In mine tailings contaminated with several metals, *P. vittata* was found to hyperaccumulate As, and accumulate Pb and antimony (Sb), with shoot concentrations reaching 41.5–4106.1, 16.8–499.5, and 12.5–321.5 $\mu\text{g g}^{-1}$, respectively. *Viola principis* was found to hyperaccumulate Cd, Pb, and As, with shoot concentrations reaching 14.3–1201, 122.3–2350, and 25.8–1032 $\mu\text{g g}^{-1}$, respectively (Wan et al. 2017).

Ultramafic soils, including serpentine soils, represent some of the most extensively studied extreme environments affecting plant growth (Harrison and Rajakaruna 2011; Hulshof et al. 2026). These soils are characterized by high levels of metals such as magnesium (Mg), Ni, Mn Co, and Cr, low levels of essential nutrients, high magnesium: calcium ratios, and a generally high pH. They are often shallow, poorly structured, and have low water-holding capacity (especially in Mediterranean climates), resulting in exposed and barren conditions (Kruckeberg 1992). Ultramafic soils are found in regions like the Appalachian Mountains (eastern North America), the Klamath-Siskiyou Mountains (western North America), the

Alps (notably, Italy), the Urals (Russia), tropical regions such as New Caledonia and Cuba, and southern Africa and Australia (Kruckeberg 2007; Rajakaruna et al. 2009; Teptina et al. 2018; Hulshof and Spasojevic 2020; Garnica-Díaz et al. 2023). Plants capable of surviving on serpentine soils, including many endemics, exhibit a suite of physiological and morphological traits that allow them to tolerate these stressful environments (Rajakaruna and Boyd 2008; O'Dell and Rajakaruna 2011; Hidalgo-Triana et al. 2023; Samojedny Jr. et al. 2023).

Plants endemic to soils derived from ultramafic rocks may exploit these habitats as refuges from competition with nearby, intolerant species. The physiological cost of tolerating the harsh abiotic conditions typical of ultramafic soils is generally thought to preclude fast-growing, competitive species from establishing there. In contrast, endemic species exhibit specialized adaptations, such as slow growth, low photosynthetic and nutrient uptake rates, low specific leaf area, and slow tissue turnover, that confer tolerance but reduce their competitive ability on more fertile soils (Chapin III et al. 1993; Kazakou et al. 2008; von Wettberg et al. 2014). Kruckeberg (1951) proposed that the occurrence of serpentine endemic plants is not due to a specific requirement for serpentine soil, but rather to their inability to compete with non-serpentine species outside these edaphic islands. While the abiotic stressors and the plant competitive dynamics characteristic of ultramafic soils are well documented, the relationship between ultramafic soils and the potentially unique animal communities they harbor is understudied. However, several studies have examined high-Ni and serpentine-endemic insects (Ehrlich et al. 1975; Boyd 2009), as well as a fish taxon restricted to pools overlying ultramafic rock (Baumsteiger and Moyle 2019).

One trait that has repeatedly evolved in serpentine-adapted plants is Ni hyperaccumulation (Kruckeberg and Reeves 1995). Although Ni is an essential micronutrient due to its role as a cofactor of urease, an enzyme responsible for hydrolyzing urea into ammonium, it becomes toxic at elevated levels (Kabata-Pendias 2001; Bhalerao et al. 2015; Ferrero et al. 2020; Ghasemi et al. 2014). While there is not a definitive single concentration at which Ni toxicity occurs, due to variation among species, soils, and exposure conditions, for most non-tolerant plants, foliar Ni concentrations above 10–50 $\mu\text{g g}^{-1}$ dry weight are considered toxic (Kabata-Pendias 2011; Chaney 2012). More than 520 plant species are known to hyperaccumulate Ni, making it the most commonly hyperaccumulated element; approximately 75% of all known hyperaccumulator taxa accumulate Ni (Baker et al. 2000; Reeves et al. 2018). Because metal hyperaccumulation imposes significant physiological costs, its persistence and maintenance suggest strong evolutionary advantages (Boyd and Martens 1992; Whiting et al. 2003; Boyd 2004, 2007, 2014).

Here, we will review existing research exploring seven non-mutually exclusive hypotheses proposed to explain the evolution of Ni hyperaccumulation: elemental defense, elemental allelopathy, drought tolerance, inadvertent uptake, enhanced reproductive fitness, incremental advantage, and metal tolerance and disposal (Table 1). We will also include some of the existing literature on hyperaccumulation of other metals in relation to these hypotheses to demonstrate similarities and

TABLE 1 | Summary of existing research supporting each hypothesis.

Hypothesis	Element	Family	Existing literatures
Elemental defense	Ni	Brassicaceae	Boyd et al. (1994), Boyd and Martens (1994), Ghaderian et al. (2000), Springer (2009), and Jhee et al. (2006)
	Se	Brassicaceae	Hanson et al. (2003) and Trumble and Sorenson (2008)
	Zn, Cd	Brassicaceae	Pollard and Baker (1997), Kazemi-Dinan et al. (2014), Gallego et al. (2016), Cabot et al. (2019), and Mohiley et al. (2020)
Elemental allelopathy/facilitation	Ni	Brassicaceae	Zhang et al. (2007), Meindl and Ashman (2015), Wipf et al. (2015), and Adamidis et al. (2016)
		Sapotaceae	Boyd and Jaffré (2001)
	As	Pteridaceae	Jaffe et al. (2017) and El Mehdi et al. (2011)
	Zn, Cd	Brassicaceae	Mohiley et al. (2020, 2021)
Drought tolerance	Ni	Celastraceae	Bhatia et al. (2005)
		Violaceae	Kachenko et al. (2011) and Quintela-Sabarís et al. (2025)
		Brassicaceae	Quintela-Sabarís et al. (2025)
		Meliaceae	
		Phyllanthaceae	
		Salicaceae	
Inadvertent uptake	Zn, Cd	Asteraceae	Saeng-ngam and Jampasri (2022)
	Ni	Brassicaceae	Meindl et al. (2021) and Scartazza et al. (2022)
Enhanced reproductive fitness	Ni	Brassicaceae	Ghasemi et al. (2014)
Incremental advantage	Ni	Brassicaceae	Assunção et al. (2003)
Metal tolerance and disposal	Cu	Lamiaceae	Reilly and Stone (1971)
	Zn	Brassicaceae	Rascio (1977)

Note: Element symbols are defined as follows: arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), selenium (Se), and zinc (Zn). See Boyd (2014) and Manara et al. (2020) for additional supporting literature on the evolution and adaptive significance of metal(loid) hyperaccumulation.

differences between what we know about Ni hyperaccumulators and other metal-accumulating species. Finally, we will highlight current gaps in our understanding that offer opportunities for future research.

Although numerous reviews have examined the adaptive significance of metal hyperaccumulation (e.g., Boyd 2007; Pollard et al. 2014; Manara et al. 2020), most have focused on individual hypotheses or single-element systems, and few have integrated recent molecular and ecological advances. Here, we provide a comprehensive synthesis of seven non-mutually exclusive hypotheses for Ni hyperaccumulation, incorporating new insights from genetics, physiology, and community ecology. In doing so, we identify several key research gaps: (1) limited understanding of the molecular regulation of Ni uptake and sequestration; (2) minimal exploration of the ecological roles of Ni in reproduction, pollination ecology, and food-web transfer; (3) lack of data on plants that accumulate but do not hyperaccumulate Ni; and (4) insufficient integration of serpentine ecology and evolutionary context into hyperaccumulation research. By addressing these gaps, we aim to provide an updated framework for understanding the multifunctional adaptive significance of Ni hyperaccumulation.

1.1 | Elemental Defense

The elemental defense hypothesis, which suggests metal hyperaccumulation provides a protective advantage, has the strongest research support, particularly for Ni hyperaccumulation. This hypothesis proposes hyperaccumulation may protect the plant from herbivores and/or pathogens. Most studies of the elemental defense hypothesis have focused on Ni hyperaccumulators (Boyd and Martens 1999). Reduced damage by herbivores and pathogens may also provide a selective advantage for the repeated evolution of serpentine tolerance (Boyd 2007).

1.1.1 | Defensive Enhancement Hypothesis

Adapted plant defense refers to traits that have evolved through natural selection to reduce damage from herbivores and/or pathogens (Boyd 2007). The defensive enhancement hypothesis proposes that some plants deter or poison herbivores through chemical defense achieved by accumulating toxic elements that herbivores cannot tolerate. This hypothesis suggests hyperaccumulation evolved by metal accumulation being defensively effective at lower concentrations, with increasing metal

concentration in turn increasing defensive effectiveness, leading to higher fitness in plants that accumulate higher concentrations of metal.

Numerous studies involving leaf-chewing herbivores have supported a defensive role for Ni hyperaccumulation (Boyd and Martens 1994; Boyd et al. 1994; Boyd and Moar 1999; Mincey and Boyd 2018). However, for other herbivore feeding modes, such as cell disruptors, there is little evidence supporting elemental defense (Boyd and Martens 1999). Additionally, two studies found hyperaccumulation had an opposite effect (resulting in increased damage to hyperaccumulating plants): one involving a pathogen and the other a snail (Boyd and Jaffré 2001; Hanson et al. 2003).

Vesk and Reichman (2009) concluded high metal concentration in hyperaccumulators generally deterred herbivory by terrestrial insects, but there was variation in tolerance among species, and this pattern was not present in snails. Variation in study outcomes is likely influenced by differences in Ni concentrations across tested plant species, as well as varying levels of metal tolerance among natural enemies. Ni-based defense against antagonists typically becomes ecologically effective at foliar Ni concentrations of approximately $300\text{--}1000\text{ }\mu\text{g g}^{-1}$ dry weight, with strong deterrence or toxicity reported above $1000\text{--}3000\text{ }\mu\text{g g}^{-1}$ (Boyd and Martens 1998; Jhee et al. 2005; Boyd 2007). Many Ni hyperaccumulators exceed these levels, often reaching $>10,000\text{ }\mu\text{g g}^{-1}$ (Reeves et al. 2018), well within or beyond the range associated with defensive effects.

The persistence and further escalation of metal accumulation beyond concentrations already effective for defense may reflect multiple evolutionary and physiological drivers. First, continual coevolution with increasingly metal-tolerant herbivores and pathogens may select for progressively higher metal concentrations, creating an evolutionary arms race (Endara et al. 2017). Second, synergistic benefits with other selective pressures, such as enhanced drought tolerance, nutrient acquisition, or allelopathic interactions, may reinforce selection for higher accumulation. Third, the physiological mechanisms responsible for uptake and sequestration may be difficult to fine-tune, leading to inherently high accumulation once the trait evolves. Finally, elevated metal concentrations may confer secondary benefits, such as improved reproductive success or competitive ability, that outweigh the metabolic costs of maintaining hyperaccumulation. Together, these factors could explain why metal accumulation continues to increase even when lower concentrations are already ecologically effective.

This coevolutionary dynamic can be investigated in several Ni-tolerant herbivores that have adapted to feed exclusively on hyperaccumulator hosts. *Melanotrichus boydi* (Hemiptera: Miridae) and *Chrysolina pardalina* (Coleoptera: Chrysomelinae) are insect species that tolerate high Ni levels while feeding exclusively on the Ni hyperaccumulators *Streptanthus polygaloides* (Brassicaceae) and *Berkheya coddii* (Asteraceae), respectively (Boyd 2014). *Chrysolina pardalina* is able to tolerate Ni via efficient Ni elimination from its gut (Przybyłowicz et al. 2003). Przybyłowicz et al. (2003) also found Ni concentrations of $1012\text{ }\mu\text{g g}^{-1}$ in the exuviae of *C. pardalina* larvae, suggesting molting as a mechanism to eliminate Ni from the body. Nickel

concentrations in other tissues were less than $60\text{ }\mu\text{g g}^{-1}$. The exact mechanism of how *M. boydi* tolerates high levels of Ni is yet to be discovered. This tolerance mechanism is hypothesized to have evolved as an elemental defense for the insect itself, although empirical evidence remains limited (Boyd 2009).

Feeding mode can also play a key role in determining the effectiveness of elemental defense against herbivory. Jhee et al. (2005) found that leaf- and root-chewing herbivores were significantly negatively impacted by hyperaccumulation, whereas phloem feeders and cell disruptors showed more variable responses. Moreover, as Meindl et al. (2021) demonstrated, hyperaccumulators often exhibit organ-specific variation in hyperaccumulation, which may selectively deter some herbivores while leaving others unaffected. They found hyperaccumulators in the Brassicaceae had significantly higher concentrations of Ni in their leaves compared to indifferent plants (Meindl et al. 2021). Boyd et al. (2008) examined patterns in Ni hyperaccumulation in *Senecio coronatus* (Asteraceae), finding a greater concentration of Ni in the leaves compared to the roots. This organ-specific hyperaccumulation likely primarily deters leaf-chewing herbivores.

Organ-specific patterns of Ni accumulation have been observed in multiple hyperaccumulator species, often with elevated concentrations in leaves and reproductive tissues relative to roots and stems. For instance, *Senecio coronatus* (Asteraceae) and *Streptanthus polygaloides* (Brassicaceae) both exhibit higher foliar Ni concentrations compared to roots, suggesting selective allocation of Ni to tissues most exposed to herbivory (Boyd et al. 2008; Sánchez-Mata et al. 2014). Similarly, *Noccaea praecox* and *N. caerulescens* (Brassicaceae) concentrate Ni in epidermal leaf cells and floral structures (Meindl et al. 2014; Jakovljević et al. 2024). In *Pycnandra acuminata* (Sapotaceae), Ni is localized within laticifers and leaf tissues, forming part of a potential chemical defense system (van der Ent, Spiers, et al. 2024). Such spatial heterogeneity in Ni allocation aligns with the Optimal Defense Hypothesis (ODH), which posits that plants invest defensive resources preferentially in organs that are critical to fitness or more likely to experience herbivory or pathogen attack (Zangerl and Bazzaz 1992; Keith and Mitchell-Olds 2017). Therefore, organ-specific metal hyperaccumulation may represent a strategic allocation of metal-based defenses consistent with ODH predictions.

Herbivores exhibit three primary responses to Ni-rich plant tissue: avoidance, diet dilution, or tolerance. Some insects have coevolved with hyperaccumulator species and developed mechanisms for tolerating Ni. Two tolerance strategies include immobilization of Ni in the gut to prevent uptake and metal accumulation within the insect (Boyd et al. 2006). For example, *M. boydi*, which feeds exclusively on *S. polygaloides*, accumulates over $700\text{ }\mu\text{g Ni g}^{-1}$ dry mass (Wall and Boyd 2006).

It is currently unknown whether hyperaccumulator insects experience any metabolic benefits from Ni accumulation. However, Ni accumulation in insects has sometimes been found to deter predation. Predators fed *M. boydi* responded variably, with three out of four accumulating Ni to $420\text{--}470\text{ }\mu\text{g g}^{-1}$. One predator, *Misumena vatia*, experienced lower survival rates when fed *M. boydi*, suggesting a potential defensive effect of

Ni hyperaccumulation against *M. vatia*, though this defense was not effective against all predators (Boyd and Wall 2001). When *Podisus maculiventris*, a generalist predator, was fed Se-accumulating herbivore *Spodoptera exigua*, it experienced slower progression through each developmental stage, higher mortality rates, and 20% less body mass at the adult stage. This study demonstrates the potential for metal accumulation as a mechanism to deter predation in accumulator insects (Vickerman and Trumble 2003). The potential fitness benefits of metal accumulation in insects should be further researched to develop a better understanding of this trait.

One ecological implication of herbivory on hyperaccumulators is the potential for metal mobilization through a food web (Gall et al. 2015) as Ni-tolerant herbivores and pollinators may transport metals to other ecosystem components. For instance, *Apis mellifera* and *Bombus vandykei* (Hymenoptera: Apidae), both floral visitors to *S. polygaloides*, had elevated whole body Ni concentrations compared to conspecifics foraging on non-hyperaccumulator plants (Wall and Boyd 2002).

Nickel hyperaccumulation may also reduce seed predation. *Tribolium confusum* (Coleoptera: Tenebrionidae), a granivorous beetle, suffered higher rates of mortality when fed *S. polygaloides* seeds containing Ni concentrations below 1000 $\mu\text{g Nig}^{-1}$ compared to individuals fed seeds of non-hyperaccumulators. This demonstrated the ability of hyperaccumulators to deter herbivory even before germination (Mincey et al. 2018).

Nickel can also defend against some pathogens, including fungi, bacteria, and viruses, but this question has been rarely examined. One study investigating pathogen resistance in Ni hyperaccumulators tested *S. polygaloides* grown in either low-Ni soil or NiCl_2 -amended soil (Boyd et al. 1994). Plants in the high-Ni treatment effectively resisted infection by the biotrophic fungus *Erysiphe polygoni* (Erysiphaceae), while those grown in low-Ni conditions showed infection. High Ni levels also correlated with increased biomass and flowering in *S. polygaloides*, suggesting both protective and growth-enhancing effects (Boyd et al. 1994). The same study tested the bacterial pathogen *Xanthomonas campestris* pv. *campestris* (Xanthomonadaceae), which causes black rot in Brassicaceae. Disease symptoms were observed only in the low-Ni treatment group and the pathogen was shown to be Ni-sensitive in vitro.

Additional evidence suggests that high-Ni soils may protect seedlings from pathogens. *Odontarrhena serpyllifolia* and *Odontarrhena chalcida* (Brassicaceae) inoculated with *Pythium mamillatum* and *Pythium ultimum* (Pythiaceae)—fungi associated with damping-off disease—showed reduced mortality as soil Ni concentrations increased (Ghaderian et al. 2000).

As for other hyperaccumulated metals, Zn has been linked to both pathogen and herbivore defense (Cabot et al. 2019). In one study, *Noccaea caerulescens* grown in Zn amended soil deterred feeding by locusts, slugs, and caterpillars, all of which preferred plants grown in low-Zn soil (Pollard and Baker 1997). *Noccaea caerulescens* appears to depend on hyperaccumulation of Zn for pathogen defense. Fones et al. (2013) proposed accumulation of metals can lead to the production of reactive oxygen species (ROS), resulting in the development of ROS tolerance in metal

hyperaccumulators. ROS production signals pathogen defense responses, and high ROS tolerance can lead to dampening of this signaling mechanism. As a result, ROS signaling has likely become uncoupled from the pathogen defense response, leaving the plant dependent on metal accumulation as a pathogen defense, kickstarting an evolutionary arms race between metal accumulating plants and metal tolerant herbivores (Fones et al. 2013).

Selenium (Se) has also been shown to protect plants against Se-sensitive fungi. However, some Se-tolerant fungi and pathogens thrive on Se hyperaccumulators, limiting the effectiveness of Se in certain contexts (Schiavon and Pilon-Smits 2017; Pilon-Smits 2019). Similarly to Ni, Se hyperaccumulation has been found to deter a variety of herbivores with different feeding strategies. However, also similar to Ni, Se hyperaccumulators remain vulnerable to certain coevolved, Se-tolerant insect herbivores. These insects may either exclude Se from absorption by the gut or exhibit broad tolerance to elevated Se concentrations. Although pollinators appear unaffected by high Se levels, experiments show they can incorporate Se into their tissues and carry Se-rich pollen, suggesting potential long-term effects that require further investigation (Schiavon and Pilon-Smits 2017).

1.1.2 | Joint Effects Hypothesis

Elemental defense differs from most plant chemical defenses, which involve specialized metabolites (Bennet and Wallsgrove 1994). Unlike specialized metabolites, which are synthesized by a plant, elemental defenses involve uptake of elements from the soil that are then sequestered in plant tissues. Being elements, they cannot be degraded by herbivores, even by those adapted to feed on the plant, whereas specialized chemicals are more susceptible to such coevolutionary counterdefenses (Boyd 1998). Just like non-accumulating plant species, hyperaccumulators also synthesize a plethora of organic defenses (e.g., specialized metabolites) to chemically fend off antagonists (Davis and Boyd 2000). The joint effects hypothesis proposes metals and organic defensive compounds can work together to enhance plant defense.

According to this hypothesis, combined effects of metals and organic compounds provide additive or even synergistic protection, allowing metals to be effective at lower concentrations when paired with organic defenses (Putra and Müller 2023). This interaction lays the groundwork for the hypothesis that hyperaccumulation may have evolved through a gradual increase in metal accumulation, driven by progressively stronger defensive benefits as metal concentrations rose (Boyd 2007). Joint effects mean that plants could begin to gain defensive advantages even before reaching hyperaccumulation thresholds.

In a study on *S. polygaloides* and its defense against the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae), plants grown in Ni-amended soil exhibited high levels of Ni but no increase in total organic defense compounds. Despite this, moths laid significantly fewer eggs, leading to fewer larvae and pupae, resulting in reduced leaf damage overall (Jhee et al. 2006). To explore whether *P. xylostella* was detecting Ni or a different cue, the researchers conducted a series of foil assays.

Moths laid more eggs on foil treated with low-Ni plant extracts, suggesting they were not directly detecting Ni but instead responding to other cues.

Interestingly, while total organic compound levels remained constant, high-Ni plants had lower concentrations of allyl glucosinolates (specifically, sinigrin). This indicates that *P. xylostella* may detect and avoid plants based on specific organic compounds rather than metal concentration directly (Jhee et al. 2006).

A comparative study between a Ni hyperaccumulator, *Odontarrhena serpyllifolia*, and non-hyperaccumulator *Alyssum simplex* found the herbivore *Tribolium castaneum* was deterred equally by both plants. When given a choice, *T. castaneum* consumed the control artificial diet disks at significantly higher frequencies than either of the plant-amended disks. This suggests that while Ni may play a defensive role in *Odontarrhena serpyllifolia*, organic compounds also contribute to herbivore deterrence (Vilas Boas et al. 2014).

Together, these observations suggest that the Joint Effects and Trade-Off Hypotheses (1.1.3) may not be mutually exclusive; rather, they likely represent different points along a continuum of defense investment, where plants may shift between synergistic and compensatory strategies depending on resource availability, metal load, and ecological context.

1.1.3 | Trade-Off Hypothesis

According to the trade-off hypothesis, using soil-derived elements for defense may be more metabolically efficient than synthesizing specialized metabolite defenses, which may require greater energy investment by a plant (Boyd 1998; Tolrà et al. 2001; Jhee et al. 2006). In a recent review, Putra and Müller (2023) advanced the elemental defense hypothesis by integrating the role of plant chemodiversity, exploring how the diversity of chemical compounds produced by plants interacts with elemental defenses to deter herbivores and pathogens. They propose that considering both inorganic and organic defense mechanisms provides a more comprehensive understanding of elemental defense and associated hypotheses, as well as the evolutionary and ecological implications of plant defense mechanisms.

1.2 | Elemental Allelopathy/Facilitation

Plants influence the soils they inhabit in several ways—modifying them through nutrient uptake while also enriching them via decomposition of plant tissues. Elemental allelopathy refers to the release of metals or metalloids from decomposing plant tissues, which subsequently interfere with the fitness of neighboring organisms.

Nickel hyperaccumulator plants may leverage metal-rich degraded tissue to suppress the germination of competitors beneath their canopies. This exclusion strategy may increase access by a hyperaccumulator to resources (light, space, and nutrients) by actively altering the soil environment through extracting metals

from deeper soil layers and depositing them in surface horizons, creating a more toxic topsoil than originally derived from the parent material.

Nickel hyperaccumulation may also affect interactions with other hyperaccumulators that benefit from elevated Ni levels, as well as influence a plant's relationships with soil microflora and mycorrhizae (Boyd and Martens 1998). In a study investigating decomposition rates and Ni release from leaf litter containing high Ni leaves, high Ni substrate was produced (Adamidis et al. 2016). This was able to be broken down by the serpentine decomposer community, releasing Ni relatively quickly. This indicates non-tolerant neighboring plants could experience elevated Ni, supporting the elemental allelopathy hypothesis. On non-serpentine soils, the low-Ni parts of the litter were selectively decomposed, indicating the effect of hyperaccumulation in litter is weaker on non-serpentine soils (Adamidis et al. 2016).

However, a challenge to the elemental allelopathy hypothesis is that many hyperaccumulated metals require extremely high concentrations to become toxic to plants (Morris et al. 2008). Therefore, the effectiveness of elemental allelopathy depends on having very high metal levels in leaf litter and surface soil layers. Another limitation is that hyperaccumulators are typically found in already metal-rich soils, where local competitors have some degree of metal tolerance. In such cases, further enrichment may not significantly impair competitors' reproductive success.

Boyd and Jaffré (2001) conducted a study on the well-known Ni hyperaccumulator *Serbertia acuminata* (now, *Pycnandra acuminata*; Sapotaceae) in New Caledonia. They investigated whether Ni phytoenrichment of soil occurred under *P. acuminata* trees. Litter, surface soil, and deeper soil samples were collected from beneath the canopies of both *P. acuminata* and nonhyperaccumulating trees. The results showed a significant difference in soil Ni enrichment under the hyperaccumulators relative to non-hyperaccumulating species, but there was no significant difference between deeper soil samples. An alternative explanation for this study's result is that *P. acuminata* seedlings preferentially establish in microsites naturally high in Ni due to the soil's parent material. However, this study found higher Ni concentrations in shallow rather than deep soil horizons, supporting phytoenrichment as the likely cause.

Randé et al. (2024) examined interactions between elemental allelopathy and the canopy effect in Zn-, Pb-, and Cd-hyperaccumulators. This study explored the balance between two opposing plant-plant interactions in metalliferous soils: (1) elemental allelopathy—where metal-rich litter from hyperaccumulating plants releases toxic metals that inhibit neighboring plants—and (2) canopy facilitation—where nurse plants improve the local microclimate, benefitting neighbors. The authors studied two metallophyte species, *Hutchinsia alpina* (Brassicaceae; high metal accumulator) and *Arenaria multicaulis* (Caryophyllaceae; low metal accumulator), in a Zn- and Cd-polluted valley in the French Pyrenees. They tested effects on two ecotypes of *Agrostis capillaris* (Poaceae) (one metal-tolerant, one less tolerant) across habitats with high and low soil metal contamination. Results showed that elemental allelopathy from *H. alpina* negatively affected the survival of the less

metal-tolerant *Agrostis* ecotype in low-polluted habitats, but no allelopathic effect was found for *A. multicaulis*, the low metal accumulator. However, both metallophytes provided significant canopy facilitation, particularly under drought conditions during an exceptionally dry year, with overall positive net effects on target plant survival. Thus, even when elemental allelopathy was present, its negative effects were outweighed by the stronger positive influence of canopy facilitation. These findings highlight that facilitation may outweigh allelopathy under stressful conditions such as drought, while elemental allelopathy may still influence competitive interactions in less stressful climates. During a year with higher precipitation, we might expect to observe an increased influence of elemental allelopathy on *A. capillaris* due to the lower dependence on the metallophytes for canopy facilitation.

A study on *Pteris vittata* (Pteridaceae), an As hyperaccumulator, offered strong support for the elemental allelopathy hypothesis due to the toxicity of As at low concentrations (Jaffe et al. 2017). Unlike other hyperaccumulators, *P. vittata* does not require high-As soils, and neighboring plants are unlikely to be As-tolerant. Elevated As levels under its canopy reduced competitor germination and growth, suggesting a possible fitness advantage. However, As levels varied among sites and individual ferns, implying that environmental conditions also influence this effect. Not every site showed significantly higher As levels under the hyperaccumulator canopy compared to surrounding soils.

Elemental allelopathy is likely most effective when hyperaccumulators colonize new, low-metal areas where native competitors lack metal tolerance. In such environments, even modest increases in surface soil metal concentrations may disproportionately benefit the hyperaccumulator. In contrast, adding more metals to already enriched surface soils may have a reduced fitness payoff.

Mohiley et al. (2021) investigated how competition influences root behavior in *Arabidopsis halleri* (a Zn and Cd hyperaccumulator). Their “split-root” experiment showed that plants placed in both high and low metal soils enhanced their metal uptake, especially those from nonmetalliferous origins that had greater Zn and Cd content when exposed to competition for light. This suggests that metal accumulation can be enhanced by competitive pressure and is therefore not entirely genetically predetermined. However, the underlying mechanisms of metal uptake and tolerance are genetically encoded and must already be present to enable metal accumulation in these plants.

1.2.1 | Elemental Facilitation

Elemental facilitation posits that while phytoenrichment of soil by hyperaccumulators can inhibit the growth of less metal-tolerant species beneath their canopies, it may also promote the germination and enhanced growth of metal-tolerant species. In Se hyperaccumulator systems, soils beneath plant canopies have been found to contain significantly higher Se concentrations compared to soils from nearby areas without hyperaccumulators. Elevated Se levels have been shown to inhibit the germination and growth of Se-intolerant species, often resulting

in reduced canopy cover around hyperaccumulators. Selenium also appears to facilitate the establishment and growth of Se-tolerant species near these plants (El Mehdawi et al. 2011; Schiavon and Pilon-Smits 2017).

El Mehdawi et al. (2011) found that Se hyperaccumulation by *Artemisia ludoviciana* and *Sympyotrichum ericoides* (both Asteraceae) may be facilitated by the presence of other Se hyperaccumulators. At sites where these species exhibit Se hyperaccumulation, they are frequently observed growing in close proximity to other hyperaccumulators. When growing near these neighboring species, *A. ludoviciana* and *S. ericoides* showed a two-fold increase in biomass and a 10- to 20-fold increase in Se concentrations.

This observed facilitation may be due to a priority effect, in which the first hyperaccumulators to colonize a Se-rich site modify the soil chemistry, creating favorable conditions for other Se-tolerant species. This pattern would instead be explained by a historical sequence of colonization rather than purely elemental facilitation (Fukami 2015).

1.2.2 | Elemental Allelopathy via Pollen

It has been hypothesized that Ni accumulation in pollen may contribute to elemental allelopathy through shared pollinators among co-flowering plant species. Wipf et al. (2015), investigating this phenomenon in *S. polygaloides*, found pollen accumulated Ni in proportion to soil Ni levels and contained significantly higher concentrations than the pollen of the nonaccumulator *Mimulus guttatus* (now, *Erythranthe guttata*; Phrymaceae). When *M. guttatus* received pollen from *S. polygaloides*, its pollen germination rate increased; however, the proportion of pollen tubes reaching the ovary declined, resulting in reduced seed set. Interestingly, higher concentrations of Ni accumulation in the pollen did not intensify these effects. Further field studies involving a broader range of hyperaccumulator taxa are needed to better evaluate this potential mechanism of elemental allelopathy via pollen.

The hyperaccumulation of Ni in flowers may inadvertently result in an “elemental filter” (Meindl and Ashman 2015). Elemental filtering suggests that metal hyperaccumulation may reduce pollinator visitation rates and richness, while also altering pollinator species composition, compared to nearby, closely related non-hyperaccumulating species. In natural populations of *Streptanthus* (*S. polygaloides* and *S. tortuosus*), one hyperaccumulator (*S. polygaloides*) and one non-hyperaccumulator (*S. tortuosus*), differing floral visitation rates were observed (Meindl and Ashman 2015). Although the richness of pollinators for each species was not significantly different, each species hosted distinct pollinator communities, potentially contributing to pollinator-mediated reproductive isolation.

Metal hyperaccumulation in reproductive structures may influence the reproductive efficiency of these species as well as their interactions with seed predators and dispersers. Elevated metal concentrations have been associated with increased flowering, enhanced pollen viability, and improved seed germination in hyperaccumulators (Ghasemi et al. 2014; Meindl

et al. 2014). However, hyperaccumulation in floral rewards can also have ecological implications, such as transfer of metals into food webs (Gall et al. 2015). For example, Ni transfers from flowers of *Hybanthus austrocaledonicus* (Violaceae) to *Apis mellifera*, the common European honeybee (Boyd et al. 2006). This metal transfer may negatively affect pollinators, potentially resulting in reduced reproductive success due to pollinator limitation. Nonetheless, some hyperaccumulators appear to mitigate this risk. For instance, *Odontarrhena* species (Brassicaceae) show significantly lower Ni concentrations in pollen compared to the rest of the stamen, potentially preserving pollinator interactions and reproductive fitness (Pavlova et al. 2016).

This elemental filter may be adaptive by preventing gene flow between hyperaccumulators species on metalliferous soils and other populations from non-metalliferous soils. This hypothesis has not been directly tested but could have implications for eventual divergence of species that have both hyperaccumulating and non-hyperaccumulating populations. This raises the question of whether similar trends could be observed in non-hyperaccumulating, serpentine-tolerant species (Boyd 2004).

Although the *Elemental Filtering* and *Enhanced Reproductive Fitness* hypotheses may appear contradictory, they likely describe different stages or dimensions of the evolutionary trajectory of hyperaccumulation. Enhanced reproductive fitness may arise from physiological or nutrient-use advantages associated with metal uptake, such as improved N metabolism or increased flowering (Ghasemi et al. 2014; Scartazza et al. 2022), which elevate individual reproductive output under metal-rich conditions. In contrast, elemental filtering acts primarily through ecological and reproductive isolation: high metal concentrations in reproductive tissues may reduce pollinator diversity or shift pollinator assemblages, limiting gene flow with non-hyperaccumulating populations. Rather than opposing each other, these processes could in fact be complementary, where metal accumulation may enhance reproduction within hyperaccumulating populations while simultaneously promoting reproductive isolation from non-hyperaccumulating populations. This framework reconciles the physiological and ecological roles of metal accumulation in shaping both fitness and diversification.

1.3 | Drought Tolerance

The drought tolerance hypothesis suggests hyperaccumulated Ni may help plants respond to drought stress. Water is typically moved from the soil into a plant's xylem by accumulating solutes, which increases solute concentration inside root hairs, lowering water potential, thereby facilitating water movement from soil into roots. From there, water is drawn up through the xylem due to negative hydrostatic pressure created by transpiration. In dry soils, where water uptake into roots can be challenging, plants may take up metals to increase cellular osmotic potential (thereby reducing cellular water potential), which helps in this water absorption process. Thus far, the few studies that have examined the relationship between Ni hyperaccumulation and drought stress have not found results that support this hypothesis.

A study examining the relationship between metal hyperaccumulation and leaf economics compared chemical leaf traits between five hyperaccumulator species and ten nonhyperaccumulator species in Sabah, Malaysia (Quintela-Sabarís et al. 2025). While Ni hyperaccumulation was not linked directly to the leaf economics spectrum, it was found to be associated with carbon isotope discrimination and K concentrations. This suggested that low water availability may induce hyperaccumulation behavior, supporting the hypothesis that metal hyperaccumulation plays a role in drought tolerance via osmoregulation.

In another experiment, *Hybanthus floribundus* subsp. *floribundus* (Violaceae), a Ni hyperaccumulator shrub native to Australia, was grown in Ni-amended soil and subjected to several drought treatments to investigate the role of hyperaccumulation in drought tolerance (Kachenko et al. 2011). The study found no significant changes in growth rate, relative water content, gas exchange rate, or carbon isotope discrimination in response to drought stress. Although plants exhibited water use efficiency, suggesting tolerance to drought, Ni concentrations did not significantly differ between treatment groups, indicating that Ni hyperaccumulation did not play a direct role in osmotic adjustment.

Similarly, the Australian Ni hyperaccumulator and serpentine endemic *Stackhousia tryonii* (Celastraceae) was studied to determine whether hyperaccumulation contributed to osmotic adjustment under drought stress (Bhatia et al. 2005). Plants were subjected to various water stress treatments, and biomass and shoot Ni concentrations were measured. Water stress significantly affected shoot growth, with Ni concentration in shoots more than twice as high under 20% field capacity (FC) compared to 100% FC. As water availability increased, Ni concentrations decreased. The authors concluded that Ni plays a facultative role in osmotic adjustment, suggesting that although Ni helps with water uptake under drought conditions, its concentration does not change significantly in response to water availability.

Controlled drying of soil is a common experimental approach used to test drought tolerance, with researchers maintaining specific percentages of a soil's field capacity (FC) using irrigation to simulate consistent drought conditions (e.g., Bhatia et al. 2005). Another method involves using polyethylene glycol (PEG) to simulate drought conditions. This method was used to compare the effects of Ni and drought between *Cleome heratensis* (Cleomaceae), a serpentine endemic, and *Cleome foliolosa*, a closely related non-serpentine tolerant species (Eskandari et al. 2017). Nickel presence improved growth and osmoprotectant synthesis, and decreased oxidative stress, in *C. heratensis* exposed to PEG-simulated drought compared to *C. foliolosa*. Additionally, a study on *Chromolaena odorata* (Asteraceae), tested the effects of PEG-induced drought stress on Zn and Cd accumulation (Saeng-ngam and Jampasri 2022). *Chromolaena odorata* is a metal accumulator; however, it is not considered a metal hyperaccumulator. Plants were grown in a 20% Hoagland solution enriched with different concentrations of PEG and metals. The study concluded that Cd and Zn accumulation were not significantly affected by the presence of PEG. These results do not support the drought tolerance hypothesis, though they suggest that *C. odorata* may be useful for phytoremediation in areas experiencing high drought stress.

1.4 | Inadvertent Uptake

The inadvertent uptake hypothesis, proposed by Boyd and Martens (1992), suggests that metal hyperaccumulation is an unintended consequence of hyper-efficient, non-specific nutrient scavenging in serpentine-tolerant plants that grow in nutrient-deficient soils. A study by Meindl et al. (2021) tested this hypothesis by growing nine species of Brassicaceae with varying serpentine affinities in a common garden experiment. The soils were enriched with Ni, and the researchers analyzed vegetative and reproductive organs to determine whether the uptake of metals was influenced by the soil, plant organ, or element. They found significant differences in potassium (K) concentrations, with hyperaccumulators taking up significantly more K than any other group. Soil affinity also influenced Co accumulation, with hyperaccumulators accumulating over 80% more Co than other groups. However, no significant effect of Ni on phosphorus (P), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), molybdenum (Mo), zinc (Zn), or chromium (Cr) was observed across all organs.

The increased uptake of K could be particularly advantageous for hyperaccumulator plants growing on K-deficient serpentine soils, as K is crucial for drought stress tolerance and stomatal regulation (Ameen et al. 2024). Overall, hyperaccumulators did take up more nutrients than other plant groups, but the uptake was often organ-specific. For instance, Cu concentrations were significantly higher in the leaves of hyperaccumulators, despite no significant increase in Cu across the entire plant. Since Cu is critical for photosynthesis, increased uptake in leaves may provide an advantage for hyperaccumulators. Similarly, Mn and Zn were more concentrated in anthers, as Mn is essential for pollen viability (Sharma et al. 1991) and Zn influences anther size, pollen grain size, pollen viability, and pollen-pistil interactions (Pandey et al. 2009). These findings suggest that metal hyperaccumulators not only take up more nutrients but also allocate them specifically to organs where they are most needed.

Metal hyperaccumulators may access limiting nutrients by tolerating high concentrations of toxic metal cations in soil. The proposed mechanism for this strategy is upregulation of transporters responsible for both metal and nutrient uptake (Deng et al. 2018; Ferrero et al. 2020). Nickel hyperaccumulators absorb Ni primarily as Ni^{2+} , via low-affinity transport mechanisms of divalent micronutrient elements such as Cu, Fe, and Zn. There have not yet been any high-affinity Ni influx transporters identified. The difference between Ni transport into the roots in hyperaccumulators versus non-accumulating plants is not well understood (Deng et al. 2018).

Ferrero et al. (2020) further proposed that the inadvertent uptake of Ni and other metals may result from the broad substrate specificity of several transporter families, including ZIP (ZRT/IRT-like proteins), NRAMP (Natural Resistance-Associated Macrophage Proteins), and IRT (Iron-Regulated Transporter) proteins. These transporters, originally evolved for essential micronutrients such as Zn^{2+} , Fe^{2+} , and Mn^{2+} , can also facilitate Ni^{2+} uptake due to similar ionic radii and charge. In hyperaccumulators, constitutive or enhanced expression of such transporters could unintentionally increase Ni uptake even when not directly adaptive. Over evolutionary time, these non-specific

uptake systems may have been co-opted or fine-tuned, giving rise to the hyperaccumulation trait observed today. This supports the notion that metal hyperaccumulation may have originated as a physiological byproduct of nutrient acquisition mechanisms, which later gained adaptive significance through selection for tolerance and sequestration.

Belloeil et al. (2025) found that only a limited number of metal transporters are associated with Ni hyperaccumulation in *Noccaea caerulescens*. This study provides genetic evidence that Ni hyperaccumulation in *N. caerulescens* arose through the co-option of broad-specificity micronutrient transporters for Ni uptake, in combination with enhanced vacuolar sequestration mediated by NcIREG2. These findings support the hypothesis that Ni hyperaccumulation initially evolved as a byproduct of nutrient acquisition driven by high, constitutive expression of multiple metal transporters, and subsequently acquired adaptive significance in ultramafic soils (Belloeil et al. 2025). While little is known regarding the physiological mechanisms of Ni uptake and sequestration, there has been extensive work investigating the molecular mechanisms of Zn and Cd hyperaccumulation in Brassicaceae. In species such as *Arabidopsis halleri*, several ZIP-type genes are highly expressed compared to non-accumulators, which may contribute to increased metal hyperaccumulation (Krämer 2010). Additionally, the QTL for Zn and Cd hyperaccumulation contains AhHMA4 (Heavy Metal ATPase 4). High AhHMA4 is associated with increased transcript levels of the Zn deficiency response genes in *A. halleri*, which may contribute to high Zn uptake rates (Krämer 2010).

Nickel hyperaccumulators, for example, often have high concentrations of Ca, K, and P in their tissues, even when these nutrients are scarce in soil, lending support to this hypothesis (van der Ent et al. 2018). van der Ent et al. (2018) also found that serpentine affinity influences nutrient uptake in non-hyperaccumulators. Plants with no strict serpentine affinity (indifferent plants) had the lowest concentrations of elements compared to hyperaccumulators and indicator species. While hyperaccumulators did not always have higher concentrations of all nutrients than non-accumulating endemics, they consistently had higher concentrations than indifferent plants (those found on and off serpentine soils). This suggests that plants restricted to low-nutrient soils, even if not hyperaccumulators, possess adaptations that allow them to more effectively take up limited nutrients compared to less restricted plants.

In contrast, a “split-root” experiment investigating root foraging for Ni in *S. polygaloides* found plants grew higher root biomass in Ni-rich soils, which suggests an adaptive advantage to hyperaccumulation for this species. These results suggest metallophilic root foraging, as has also been shown for Zn and Cd hyperaccumulators (Haines 2002; Liu et al. 2010), implying an active preference for metal-rich substrate, opposing the hypothesis that hyperaccumulation is a result of passive metal uptake (Mincey and Boyd 2024).

Recent research has related the hyperaccumulation of Al, Cd, Mn, and REEs to phosphorus (P) scavenging. These elements are mobilized by functionally similar compounds that are used by plants to mobilize P (Lambers et al. 2022). Hyperaccumulation

in these species may not be an adaptation, but instead a result of P scavenging mechanisms (Lambers et al. 2022).

De Groote et al. (2018) found *Phytolacca americana*, a species that hyperaccumulates Mn on and off Mn-rich soils, secretes acids into the rhizosphere to increase phosphate acquisition, which may inadvertently increase Mn uptake. They grew plants with agar containing bromocresol with indicator dye applied to the roots. Compared to non-accumulators, the rhizosphere of *P. americana* was significantly more acidified. Additionally, concentrations of Mn in *P. americana* foliage were highest in more acidic soils, suggesting that Mn hyperaccumulation in *P. americana* is a byproduct of phosphorus scavenging in this species (De Groote et al. 2018). Varieties of *P. americana* also hyperaccumulate REEs. P-deficient soil induced secretion of organic acids by the roots and increased REE hyperaccumulation (Liu et al. 2023).

While there is no evidence of P scavenging leading to hyperaccumulation of Ni, the above studies demonstrate that hyperaccumulation may not necessarily be an adaptive trait. Instead, it may be the result of an adaptive trait directed toward macronutrient acquisition. This concept is relevant in the context of hyperaccumulation of other elements because it demonstrates the potential importance of root exudates for hyperaccumulation, which has been debated heavily in the literature (Pollard 2023). In light of these findings, the inadvertent uptake hypothesis should be investigated further to determine whether root exudates should be considered an essential step in the process of hyperaccumulation. The inadvertent uptake hypothesis is a potential explanation for the evolutionary origin of metal hyperaccumulation. Even if it currently serves as an adaptive function in hyperaccumulator plants, the trait may have first occurred as a byproduct of essential nutrient acquisition (Boyd and Martens 1992). Additionally, many of these studies have examined members of the Brassicaceae, while there are plenty of hyperaccumulator species from other families that are yet to be tested (Pollard 2023).

In summary, although uptake of metals may occur inadvertently through transporters with broad substrate specificity, subsequent allocation and sequestration processes are likely to be under adaptive physiological control. In this sense, “inadvertent” describes the origin of uptake, not the fate of metals once inside the plant. After absorption, metals such as Ni can be actively chelated by ligands (e.g., histidine, nicotianamine) and directed toward specific organs or cellular compartments where they are detoxified or functionally repurposed (van der Pas and Ingle 2019; Ferrero et al. 2020). Thus, the Inadvertent Uptake Hypothesis does not preclude adaptive allocation; instead, it suggests that hyperaccumulation may have arisen as a byproduct of nutrient uptake but subsequently gained adaptive significance through the evolution of controlled internal metal distribution.

1.5 | Enhanced Reproductive Fitness

Plant reproductive fitness is the ability of a plant to produce flowers and viable offspring. Survival, reproduction, and growth are all metrics used to estimate plant fitness (Wadgymar et al. 2024).

Certain plants may experience increased reproductive fitness in response to high levels of metals in the soil. Several studies have reported enhanced growth of hyperaccumulator plants in metal-enriched soils (Krämer et al. 1996; Whiting et al. 2000; Ingle et al. 2005; Burrell et al. 2012), but there is limited research on the physiological mechanisms behind these observations.

The mechanisms behind this hypothesis remain poorly understood. Ni hyperaccumulating plants flower over long periods of time, posing difficulties estimating plant fitness. Reproductive benefits may also be difficult to quantify, particularly for florivore or seed predator deterrence. Many studies investigating metal hyperaccumulation do not include data on flowering, pollination, and seed set. Additionally, the lack of experimental studies investigating the physiological and genetic mechanisms of Ni hyperaccumulation may contribute to our lack of understanding of this hypothesis.

Ghasemi et al. (2014) demonstrated that Ni can enhance flowering in the Ni hyperaccumulator *Alyssum inflatum* (Brassicaceae). In their study, *A. inflatum* seeds were collected from Ni-rich serpentine soils in western Iran and sown under climatically controlled greenhouse conditions. Plants were treated with NiSO_4 to achieve shoot Ni concentrations similar to those observed in hyperaccumulators in the field while the effects of a high Mg treatment, another common characteristic of serpentine soils, were also included in this study.

Treatments with higher Ni concentrations significantly increased both likelihood of flowering and number of inflorescences produced. Inflorescence length and number of open flowers also increased compared to Mg and control treatments. These findings led to the hypothesis that Ni stimulates flowering, thereby enhancing reproductive fitness in this Ni hyperaccumulator. Importantly, plant size did not differ between treatment groups, suggesting that Ni primarily elevated reproductive fitness without affecting growth rates, a result consistent with previous studies by Ghasemi and Ghaderian (2009).

While physiological mechanisms underlying Ni hyperaccumulation are understudied, one potential benefit is that Ni improves N acquisition and metabolism in harsh soils. This could be due to Ni's role as a cofactor for urease, an enzyme responsible for converting urea into ammonium, a form of N plants can use (Eskew et al. 1984; Ferrero et al. 2020). In nutrient-poor, Ni-rich serpentine soils, where hyperaccumulators are often found, efficient N metabolism may be important for a hyperaccumulator plant's ability to compete and thrive.

Scartazza et al. (2022) investigated the effects of Ni on N allocation to photosynthesis in three species of *Odontarrhena*. When grown hydroponically with NiSO_4 , the strong hyperaccumulator species *Odontarrhena chalcidica* increased both N accumulation and allocation to photosynthesis. This was also true for the medium accumulator species *Odontarrhena moravensis*, though it was not for the weak hyperaccumulator *Odontarrhena muralis*, which experienced a decrease in photosynthetic performance. It currently is unknown whether this pattern is due to a role of Ni in photosynthesis in hyperaccumulator plants, or if this is merely a byproduct of Ni hyperaccumulating mechanisms. Regardless, this demonstrates

the potential for Ni hyperaccumulation to influence nutrient allocation strategies in plants and highlights the species-specific differences in tolerance and adaptation to elevated Ni levels. Additionally, it is unknown whether there are limitations to the benefits of Ni in N accumulation and allocation for hyperaccumulators.

The mechanisms linking Ni hyperaccumulation to enhanced flowering remain poorly understood, but emerging evidence suggests a potential interaction with phytohormone regulation. Nickel's role as a cofactor of urease supports nitrogen metabolism, which may indirectly influence the synthesis of phytohormones such as cytokinins and auxins that promote floral initiation and development (Eskew et al. 1984; Ferrero et al. 2020). Studies on Ni exposure in both accumulator and non-accumulator species have reported changes in ethylene and abscisic acid (ABA) levels (Kachenko et al. 2011; Bhalerao et al. 2015), hormones that regulate flowering, fruit set, and senescence. Although these responses are typically viewed as stress-induced, hyperaccumulators may have co-opted Ni-mediated hormonal pathways as part of an adaptive reproductive strategy. Clarifying these interactions represents a key frontier for understanding the physiological basis of Ni-enhanced flowering. Further, research examining how Ni interacts with phytohormonal pathways could reveal whether Ni functions not only as a micronutrient but also as a signaling element influencing flowering and reproductive success in hyperaccumulators.

1.6 | Incremental Advantage

Most known hyperaccumulators are considered “obligate hyperaccumulators,” meaning they are restricted to metalliferous soils. However, some hyperaccumulators are classified as “facultative hyperaccumulators” (Pollard et al. 2014). These species grow both on metalliferous and non-metalliferous soils but, when found on metalliferous soils, hyperaccumulate metals. Facultative hyperaccumulators are particularly useful for intraspecific comparisons between hyperaccumulating and non-hyperaccumulating plants. Pollard et al. (2014) outlined three non-mutually exclusive hypotheses to explain adaptive benefits of facultative hyperaccumulation.

One such hypothesis is the *incremental advantage hypothesis*, which suggests that there is an adaptive benefit to having the physiology of a hyperaccumulator, even when many individuals of the species grow on non-metalliferous soils and do not hyperaccumulate metals. This hypothesis proposes that physiological traits associated with hyperaccumulation provide a benefit that extends to individuals growing on regular soils. For example, facultative hyperaccumulators often exhibit elevated levels of trace elements when growing on normal soils, although these levels fall short of the hyperaccumulator threshold (Assunção et al. 2003). These raised trace element levels may play a role in deterring herbivory. Coleman et al. (2005) tested the effects of various metal concentrations on the diet of diamondback moth (*Plutella xylostella*) larvae. They found that while metals at hyperaccumulator levels were lethal to larvae, even metals at accumulator levels were toxic. Specifically, Cd, Mn, Ni, Pb, and Zn were toxic at concentrations lower than those found in hyperaccumulators, with Cd and Pb being toxic near their normal

concentration range, and Zn being toxic within the normal concentration range.

This hypothesis does not limit the adaptive significance of high trace metal concentrations to defense mechanisms. Instead, it acknowledges the potential additional advantages of hyperaccumulation for nutrient acquisition and osmotic balance. It also recognizes that this physiology could cause facultative hyperaccumulators to accumulate metals to non-adaptive or maladaptive concentrations (Pollard et al. 2014). Other hypotheses for the maintenance of facultative hyperaccumulation in some species include the *inadvertent uptake hypothesis* (discussed earlier in this paper) and *phylogenetic conservatism*, in which facultative hyperaccumulators may descend from obligate hyperaccumulators (Broadley et al. 2001; Manara et al. 2020).

1.7 | Metal Tolerance and Disposal

Metal hyperaccumulation may serve as a mechanism for tolerance to metalliferous soils by allowing plants to sequester metals in leaf tissues and dispose of the metals by shedding leaves (Boyd and Martens 1992). While sequestration of metals in leaf tissues is well documented, there are few studies that directly demonstrate the adaptive significance of metal disposal through leaf shedding.

At the molecular and cellular level, once Ni is taken up via broad-specificity cation transporters from the soil, Ni is chelated by a ligand such as histidine or nicotianamine (van der Pas and Ingle 2019). The universality of these ligands is a gap in our knowledge, and chelation in a range of Ni hyperaccumulators should be studied to provide a better understanding of this process. Ni then may move through xylem though it is unclear if it is transported as a Ni-ligand complex or a free ion, and the exact transporters are currently unknown. In the xylem sap, most Ni is in the form of a free cation. In the shoot, Ni accumulates in epidermal vacuoles via IREG/FPN transporters, forming either Ni-citrate or Ni-malate complexes. The transporters involved in xylem unloading are also currently unknown (van der Pas and Ingle 2019).

For example, *Ocimum centraliafricanum* (syn. *Becium homblei*; Lamiaceae), a Cu hyperaccumulator, removes excess Cu at the end of the growing season by shedding leaves or by burning due to wildfires. This allows the plant to produce new leaves, flowers, and seeds at the start of the next growing season before Cu concentrations reach toxic levels (Reilly and Stone 1971). Similarly, Rascio (1977) found that *Nothaea cepaeifolia* (Brassicaceae), a Zn accumulator, had higher Zn leaf levels during autumn and lower levels in shoots and roots during autumn. In the spring, Zn concentration remained higher in leaves, but levels in shoots also increased compared to roots, with a significant overall reduction in plant Zn content. This suggests that Zn is being actively disposed of, likely through shedding of leaf tissues. There was no evidence of metal exclusion in this species, as roots had the lowest Zn accumulation, further supporting the idea that tolerance to metalliferous soils is achieved through disposal of leaf tissue rather than by excluding metals.

2 | Common Experimental Techniques

Most experiments testing the adaptive significance of metal hyperaccumulation in plants use amended soils to create treatment groups with varying levels of metal exposure. Soil amendment involves adding substances to alter soil properties. In many Ni physiology studies, researchers amend soil with substances that increase bioavailability of Ni or directly add Ni. Methods that increase Ni bioavailability include lowering soil pH or introducing siderophore-producing bacteria (Rajkumar et al. 2010; Ly et al. 2024). Nickel also can be introduced via Ni salts (e.g., $\text{Ni}(\text{NO}_3)_2$, NiSO_4 , and NiCl_2) at various concentrations. It can be dissolved in water and poured or fertigated into soil or added in granular form to dissolve during watering (Zhao et al. 2002; Ashraf et al. 2011).

Hydroponics are another common technique used to test plant responses to high levels of specific elements. This technique, widely used across various studies, allows researchers to grow almost any species in a nutrient solution without soil (Nguyen et al. 2016). Hydroponics is particularly useful because it enables manipulation of a single variable, which is more difficult using soil-based methods. This approach allows researchers to assess a plant's tolerance to specific trace metals, measure root and shoot concentrations of the metal of interest, and determine whether metals have specific functions. Hydroponics also provides better control of metal availability and eliminates the confounding influence of rhizosphere bacteria and fungi (van der Ent, Kopittke, et al. 2024).

However, it is important to be cautious when using hydroponics to study hyperaccumulators. It is crucial not to mistakenly attribute hyperaccumulator status to species that surpass tolerance limits, where metals may "break through" uptake barriers and be transported at high levels into shoots (van der Ent and Rylott 2024). Often, common weed species that do not hyperaccumulate metals in nature are tested with high doses of certain metals using hydroponics, leading them to appear as "hyperaccumulators." These extreme doses may cause eventual mortality, but oftentimes experimental exposure times are too short to capture this, making conclusions about hyperaccumulator ability misleading (Baker and Whiting 2002; Reeves et al. 2018; van der Ent and Rylott 2024).

Use of amended soils versus hydroponic solutions in studying metal hyperaccumulation is a subject of debate. van der Ent et al. (2015) argue that hyperaccumulator species should be studied in their natural soils to assess their ecological relevance, compared to hyperaccumulation that only occurs in soils spiked with unnaturally high levels of metal. While field experiments provide valuable insights, they are less common than greenhouse-based studies due to the limited access to ultramafic field sites, since soils derived from ultramafic rock make up less than 1% of the Earth's surface (Garnier et al. 2009). Additionally, ultramafic plant communities are often sensitive, and manipulative field studies may have long-term unpredictable ecological impacts. Furthermore, field studies often include several uncontrollable variables. We suggest the ideal approach to studying hyperaccumulation is a combination of field-based and greenhouse studies.

Recent advances in RNA-Seq technology have enabled the identification of molecular mechanisms involved in Ni

hyperaccumulation across diverse dicotyledonous species. Cross-species analysis has revealed convergent evolution of specialized metabolite synthesis and cell wall organization. In plant families, they found high expression of genes involved in phenylpropanoid compound metabolism, which have previously been associated with iron nutrition and Cd accumulation. Furthermore, they found high expression of IREG/Ferroportin transporters across Ni hyperaccumulators from diverse plant lineages (García de la Torre et al. 2021). These findings suggest that independent plant lineages have evolved similar molecular strategies for coping with Ni toxicity. Genetic techniques such as RNA-Seq provide an opportunity to study Ni hyperaccumulation in nonmodel species, allowing for comparative studies across a wide diversity of plant lineages.

Recently, gene-editing technologies such as CRISPR-Cas9 and RNA interference (RNAi) have been used to create non-hyperaccumulating variants of hyperaccumulator species (Zhao et al. 2018; Sarma et al. 2021). These tools allow researchers to investigate genes involved in metal uptake, transport, detoxification, and storage. CRISPR-Cas9 is often used for gene knockout experiments, while RNAi targets mRNA to temporarily silence specific genes. Several studies have used these technologies to uncover the genetic basis of metal hyperaccumulation. For example, CRISPR-Cas9 and RNAi were employed to study chloroplast Cd detoxification in *Sedum plumbizincicola* (Crassulaceae), a Cd hyperaccumulator (Zhao et al. 2018). Since Cd is nonessential and highly toxic, hyperaccumulation of Cd is rare (Dai et al. 2020) and these plants must have efficient detoxification mechanisms to survive.

One such mechanism involves P1B-type ATPases, also known as Heavy Metal ATPases (HMAs). For example, Zhao et al. (2018) isolated *SpHMA1* and demonstrated that it encodes a Cd transporter in the chloroplast envelope responsible for exporting Cd from the chloroplast. They found that *SpHMA1* is expressed at very high levels in leaves of *S. plumbizincicola* (200 times higher than a non-accumulating relative, *Sedum alfredii*). Researchers grew wild-type *S. plumbizincicola*, *SpHMA1*-RNAi transgenic lines, and *SpHMA1*-CRISPR-Cas9 lines in soils amended with Cd or Zn and found much higher Cd accumulation in chloroplasts of the RNAi and CRISPR-Cas9 lines (Zhao et al. 2018).

Little is known about the mutations required for the evolution of metal hyperaccumulation. Hanikenne et al. (2008) found Zn and Cd hyperaccumulation in *Arabidopsis halleri* is dependent on HMA4. Enhanced expression of this pump is due to modified *cis*-regulatory sequences and gene copy number expansion. When this gene was transferred into a non-hyperaccumulating relative, *Arabidopsis thaliana*, they observed increased Zn movement into the xylem and activation of Zn deficiency response genes, recreating a hyperaccumulation trait in a non-hyperaccumulating plant. This study provided direct genetic evidence for how this trait evolved and opens avenues for phytoremediation and biofortification (Hanikenne et al. 2008). Further research should be conducted to enhance our understanding of the mutations that have led to the underlying genetic basis of hyperaccumulation.

Additionally, researchers have proposed using hyperaccumulator species in phytoremediation by applying gene editing to

enhance their metal accumulation capabilities. CRISPR-Cas9 and insights into genes important for hyperaccumulation could improve the ability of plants to remove metals from contaminated soils, aiding in cleanup of polluted environments (Venegas-Rioseco et al. 2021; Bhattacharyya et al. 2022).

Gene-editing hyperaccumulator species is likely to change their interactions with insects and microbes. This review has discussed several ways herbivores and soil microbes that coexist with hyperaccumulators are highly specialized (Boyd 2014; De Groote et al. 2018). However, the indirect and long-term ecosystem-level consequences of deploying gene-edited hyperaccumulators for phytoremediation are highly context-dependent and warrant targeted, long-term investigation.

3 | Advances in Understanding Nickel Localization

New technologies such as micro-x-ray fluorescence (μ XRF) have greatly advanced our understanding of metal uptake and localization in hyperaccumulator plants. Species such as *Noccaea fendleri* subsp. *glauca* (Brassicaceae) and *S. polygaloides* accumulate large amounts of Ni in reproductive organs (Meindl et al. 2014; Sánchez-Mata et al. 2014). Jakovljević et al. (2024) utilized μ XRF to investigate the elemental distribution of Ni and Zn in reproductive tissues of *Noccaea praecox* and *N. caerulescens* (Brassicaceae), both hyperaccumulators of Ni, Zn, and Cd. In both species, the primary mechanism for metal tolerance was confirmed to be accumulation and sequestration in vacuoles of leaf epidermal tissue. However, significant metal accumulation was also observed in floral organs. Interestingly, both species exhibited low metal concentrations in the perianth, contrasting with earlier findings in *S. polygaloides*, where metal accumulation was concentrated in perianth tissues (Jakovljević et al. 2024).

Other researchers have applied μ XRF to study Se hyperaccumulation in *Stanleya* and *Thelypodium* species (both Brassicaceae). Cappa et al. (2014) used μ XRF and micro x-ray absorption near-edge structure (XANES) spectroscopy to analyze Se distribution and chemical speciation in both vegetative and reproductive tissues. Their study included field-collected leaves and seeds, mapping Se alongside Ca and Fe. XANES analysis provided insight into the chemical forms of Se within the plants.

Two distinct patterns of Se localization were observed among the eight species studied: half localized Se within vascular tissues, while the other half accumulated it in leaf margins. Seeds from two species (*Stanleya elata* and *Thelypodium laciniatum*) could not be analyzed using μ XRF due to low Se levels, highlighting a potential limitation of the method. XANES analysis revealed that over 50% of Se in leaves was in organic form, primarily modeled as C-Se-C compounds.

Nickel laticifers in *P. acuminata* have recently been imaged with synchrotron XRF microscopy, microtomography (XRF- μ CT), and synchrotron x-ray phase contrast imaging microtomography (PCI- μ CT). This technology was leveraged by van der Ent, Spiers, et al. (2024) to model the laticiferous system in *P. acuminata* for the first time, demonstrating Ni transport via the

laticifer network in this species and the potential of this technology to advance our understanding of the underlying physiology of Ni hyperaccumulation.

These studies highlight the powerful applications of μ XRF and XANES technologies for investigating metal accumulation and sequestration in hyperaccumulator plants. Such findings not only deepen our understanding of metal localization patterns but also contribute to broader evolutionary and ecological questions. In particular, the insights gained from elemental mapping have been used to inform phylogenetic analyses, as researchers seek to understand the evolutionary origins of Se hyperaccumulation in *Stanleya* (Cappa et al. 2014). Similar approaches could be applied to study the evolution of Ni hyperaccumulation across plant lineages.

4 | Future Research Directions

The elemental defense hypothesis is the most extensively studied among those discussed here, with substantial empirical support (Table 1). However, these hypotheses are not necessarily mutually exclusive, and significant gaps remain in our understanding of each. One notably understudied area in the field of plant elemental concentration is the role of metal accumulation when the threshold for hyperaccumulation is not met. There is limited information on the number of these accumulator species, and little is known about the extent to which the hypotheses presented, such as elemental defense, allelopathy, and enhanced reproductive fitness, apply to them. Most existing research on the elemental defense hypothesis has focused on Ni hyperaccumulators. Expanding this research to include other metal/metalloid accumulators and testing additional hypotheses could yield valuable insights (see Steven and Culver 2019). Additionally, the effects of Ni accumulation on vertebrate herbivores remain largely unexplored. Further, the elemental defense hypothesis can be expanded to include the role of plant chemodiversity (see Putra and Müller 2023). Integrating the multifunctionality of chemodiversity could enhance our understanding of the eco-evolutionary drivers behind metal hyperaccumulation. Key knowledge gaps also remain regarding how edaphic, population, temporal, and spatial factors interact to influence hyperaccumulator diversity as well as the adaptive significance of the hyperaccumulator trait, underscoring the need for broader, more integrative research frameworks.

Elemental allelopathy is likely influenced by a range of environmental factors (Jaffe et al. 2017), yet the specific abiotic and biotic conditions that promote or inhibit this mechanism have not been thoroughly investigated. Because elemental allelopathy is not universally observed among hyperaccumulators, it is likely that certain environmental conditions contribute to its success. Identifying these factors could help clarify ecological patterns associated with elemental allelopathy and its potential role in plant competition.

Another key gap in our understanding lies in the physiological mechanisms underlying Ni hyperaccumulation and the functional role of Ni within hyperaccumulator plants (van der Pas and Ingle 2019). Critical questions remain unanswered, including the identity and regulation of Ni transporters involved

in root uptake, the processes of Ni loading into the xylem and phloem, the role of Ni translocation in hyperaccumulation, and the physiological differences that distinguish hyperaccumulator species from non-accumulators (Deng et al. 2018; van der Ent, Spiers, et al. 2024). Addressing these questions will be essential for a more comprehensive understanding of the evolution and function of Ni hyperaccumulation.

To date, relatively few studies have addressed the phylogenetic origins of hyperaccumulation (Broadley et al. 2001; Manara et al. 2020). Hyperaccumulators form a polyphyletic group within angiosperms, indicating that hyperaccumulation has evolved multiple times independently, underscoring the complexity and evolutionary plasticity of this trait. Early phylogenetic analyses suggested multiple independent origins of Ni hyperaccumulation in the genus *Alyssum* (Mengoni et al. 2003; Cecchi et al. 2010). However, subsequent phylogenetic reassessments of the tribe Alysseae have reassigned most Ni-hyperaccumulating taxa previously placed in *Alyssum* to the genus *Odontarrhena*, which is predominantly composed of hyperaccumulators (Španiel et al. 2015). The few non-hyperaccumulating species within *Odontarrhena* may instead represent independent losses of the trait rather than multiple independent gains.

Answering these remaining questions requires a multidisciplinary approach via collaboration between experts in several relevant fields. Future research efforts should aim to integrate knowledge of plant physiology, molecular biology, biochemistry, ecology, evolutionary biology, and soil science to develop a clear understanding of metal hyperaccumulation and the pressures that drove the evolution of this trait. Advances in genomics and transcriptomics offer powerful tools that can be leveraged to further our understanding of hyperaccumulation, while field-based ecological studies provide context in natural environments. Collaboration between experts on various taxa, ecosystems, and geographic regions can help begin to untangle the complexity of the evolution of this trait. By bridging a variety of approaches, we can move beyond single hypothesis testing and toward a framework that reflects the likely complex role of metal accumulation in plant survival and fitness.

5 | Conclusions

Nickel hyperaccumulation is a complex adaptive strategy that enables plants to survive in harsh, Ni-rich serpentine soils. Over the past several decades, multiple non-mutually exclusive hypotheses have been proposed and tested to explain the adaptive significance of Ni hyperaccumulation (Table 1). These include elemental defense, elemental allelopathy, drought tolerance, inadvertent uptake, enhanced reproductive fitness, incremental advantage, and metal tolerance and disposal. Each of these mechanisms should be considered when studying hyperaccumulator plants. Approximately 75% of all known hyperaccumulator taxa are Ni hyperaccumulators (Baker et al. 2000; Reeves et al. 2018; Ferrero et al. 2020) and gaining a deeper understanding of their ecological and physiological traits can provide valuable insights into other hyperaccumulators, including those that accumulate other metals and metalloids. Additionally, this knowledge can enhance our understanding of plant adaptations to serpentine soils and the role these soils play in shaping plant

diversity and edaphic specialization. Finally, while the elemental defense hypothesis remains the most extensively studied, our synthesis highlights substantial gaps in understanding the multifunctionality of Ni hyperaccumulation. Specifically, future research should aim to (1) clarify physiological and molecular mechanisms underlying Ni transport and sequestration, (2) test how hyperaccumulation interacts with other selective pressures such as drought or nutrient limitation, (3) explore ecological and evolutionary consequences below hyperaccumulation thresholds, and (4) integrate genomic, transcriptomic, and field-based ecological approaches to better resolve the origins and maintenance of this trait.

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Conflicts of Interest

Nishanta Rajakaruna is an Associate Editor-in-Chief and Robert Boyd is a Guest Handling Editor but took no part in the peer review and decision-making processes for this paper.

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