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An Assessment of the Suitability of Contrasting Biosolids for Raising Indigenous Plants in Nurseries

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Abstract: Disposal of biosolids, the solid fraction of sewage treatment, is a global environmental issue. Biosolids contain valuable organic matter and plant nutrients; however, they also contain contaminants including trace elements, xenobiotics, and pathogens. The quality of the biosolids greatly depends on the source of wastewater (i.e., industrial vs. domestic) and the treatment processes. We aimed to determine the potential of three distinct biosolids and one pond sludge to grow indigenous plants for ecosystem restoration. For each amendment, we tested six indigenous species, *Veronica salicifolia*, *Corokia cheesemanii*, *Griselinia littoralis*, *Phormium tenax*, *Poa cita*, and *Cordyline australis* in bark mixed with biosolids and/or pond sludge at rates of 0–50%. There was a significant positive correlation between plant growth and biosolid addition up to a species-dependent plateau. Growth decreased at the highest rates. At a rate of 10% for fresh biosolids and 30% for aged biosolids provided consistent optimal growth across all species. The pond sludge was unsuitable for the establishment of indigenous seedlings. At the optimal rates, there were significant increases in foliar N, P, K, S, and Zn. None of the trace elements accumulated in the plants at phytotoxic concentrations or levels that presented a risk to ecosystems. Future work should determine how plants raised with biosolids perform once planted out in the field.

Keywords: biosolids; indigenous plants; nurseries; nitrogen; optimal rate; fertiliser; ecosystem restoration; seedlings

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1. Introduction

Biosolids are a treated, secondary by-product from the wastewater treatment process [1]. Continuous growth of the global population combined with increased inflows to wastewater treatment plants (WWTPs) facilities results in increasing amounts of biosolids produced [2]. Jones et al. 2021 and Di Giacomo and Romano 2022 [3,4] estimated between 360 and 380 km^3 of wastewater are generated globally every year, resulting in 45 million t yr^{-1} of sludge on a dry matter basis [5]. New Zealand (NZ) produces ca. 66,000 t yr^{-1} biosolids (363,000 t yr^{-1} fresh weight) [6].

The moisture content of biosolids ranges from 4 to 85% [7], with organic matter comprising 50 to 70% of the solid fraction [8]. Biosolids have significant amounts of plant macronutrients, especially N, P, K, S, Ca and Mg [9]. Variation of plant nutrients in biosolids are affected by age and the origin of the influent and stabilisation processes [8,10,11]. As an example, younger or fresh biosolids typically contain a higher concentration of valuable plant nutrients than older and/or composted biosolids [12,13]. Total N ranges from 1.9 to 8%, with decreasing content as the biosolids age [13]. Phosphorus concentrations average 2% [14–16]. Biosolids can have biologically significant concentrations of both essential

and non-essential trace elements (TEs) that can affect plant growth positively or deleteriously [17]. Other contaminants in biosolids include pathogens, per- and polyfluoroalkyl substances (PFAS), microplastics, pharmaceuticals, personal care products, or endocrine disruptors [2,18–20].

Current practices for biosolid management vary between countries and include incineration [21], landfilling [22], ocean discharge [6], construction materials [23], and land application [24]. The application to agricultural soil was a widely used option [9,13,15,24,25] in the early 2000s. In the EU and USA, more than 50% of the biosolids were applied to agricultural soils [26]. Land application has decreased in some European countries due to changes in environmental regulation [25]. In New Zealand, biosolid reuse is restricted by cultural and social concerns of contaminants present in the biosolids entering the food chain [27]. Therefore, some 75% of biosolids are disposed of in landfills, monofills, or discharged into the ocean, and just 3% are used in agriculture [6]. Forestry and land restoration are alternative strategies for biosolid management [28,29]. These methods not only facilitate sustainable biosolid management, but they also contribute to environmental conservation.

Biosolid reuse can extend beyond large-scale land restoration into more controlled environments such as nurseries [30,31]. The incorporation of organic waste materials, including biosolids, to replace classic growing media as peat or commercial substrate has been gaining attention since 1984 [30,32]. This approach is particularly relevant for non-food production such as ornamental or forestry plants [30,33–36]. Studies in the past have explored the benefits of this practice in nurseries, highlighting its potential as an efficient alternative for growing healthy plants [37].

The incorporation of biosolids as a part of potting mix can reduce the need for artificial fertilisers for the supplement of plant nutrients. However, the uptake of trace elements may negatively affect local ecosystems and possibly breach local regulations. High concentrations of trace elements, such as Zn, may lead to phytotoxicity [38], where the indigenous Brazilian species *Myracrodruon urundeuva*, in combination with other factors such as biosolids not being properly treated/stabilised, has led to a total plant mortality. This highlights the importance of choosing appropriate biosolids, considering the origin and appropriate application rates.

In New Zealand, many indigenous plants are adapted to low fertility soil [39,40]. Studies with biosolids in combination with low fertility soil under several application types showed that biosolids can promote plant growth at increasing rates of application; however, at rates over 12% w/w, the biosolid application may be detrimental for indigenous plants. For example, it hindered the plant growth of *Leptospermum scoparium* [41]. Similarly, Reis et al., 2017, and Seyedalikhani et al., 2019 [42,43], showed improved nutrient status and increased plant biomass after indigenous plants were grown in low fertility soils with biosolid incorporation. This also led to an increased uptake of Zn and Cd. However, the foliar concentrations were below threshold values for both phytotoxic and animal consumption. Most research has focused on incorporating biosolids to low fertility soil for potential land reforestation. However, the use of biosolids to create substrates in nurseries and their potential for seedlings has been less investigated.

In addition, given the wide variety of biosolids being produced, with contrasting chemical composition and characteristics, as mentioned previously, there is a lack of knowledge (or missed opportunity) on comparing the response of a range of indigenous species to several kinds of biosolids.

Previous research [39,41–43] has shown that increasing the concentration of contrasting biosolids will increase plant growth to a certain rate, from where higher application rates will have a deleterious effect on plant growth.

We hypothesise that the optimum rate of biosolid application will depend on the type of biosolids and the plant species. The second hypothesis is that plant nutrients and trace elements will be higher in biosolid substrates compared with substrates without biosolids. Subsequently, this will allow us to assess the nutritional status of the plants as

well as to identify the potential risks of trace element uptake by the plants at the optimal application rate.

This research aimed to identify an optimal application rate for indigenous plants based on diverse biosolids in a controlled environment, specifically, by analysing the produced dry biomass of indigenous plants. We also aimed to assess the chemical composition on leaves of indigenous NZ species.

2. Materials and Methods

2.1. Experimental Setup

Twenty-five kg of three distinct biosolids and pond sludge was obtained from various wastewater treatment plants in New Zealand. Biosolids 1 and 2 (B1 and B2) were anaerobically digested biosolids from two cities. Biosolids 3 (B3) were anaerobically digested and composted, and pond sludge (PS) was 60-year-old geobag pond sludge. Composted bark fines from Natural Bark & Compost (Foxton, Manawatu, New Zealand) were used as a substrate to be mixed with the biosolids due to their low nutrient and high moisture retention capacity [44,45] (Table 1).

Two of the biosolids were basic (B1 and B2), while two were acidic (B3 and pond sludge). The biosolids, but not the pond sludge, were rich in C, N, P, S, and K relative to the bark. All the materials contained elevated concentrations of Cu and Zn relative to the bark and to background concentrations of these elements in the soils [46].

Table 1. Properties of biosolids and bark substrate used in seedling trial, reported by LEI, 2018 [47] along with current NZ biosolids guidelines [48]. Units are in mg kg^{-1} (dw) unless otherwise indicated.

Parameter	B1	B2	B3	PS	Bark Fines	Biosolid Guidelines *
Moisture content (%)	80	21	66	39	53	-
Electrical Conductivity (mS m^{-1})	248	618	419	54.5	13.2	-
pH	8.1	7.2	6.4	4.2	5.6	-
Organic matter (%)	72	75	39	8.1	58	-
Total Organic Carbon (%)	34	39	20	3.1	23	-
Total Nitrogen (%)	6	4.9	1.89	0.35	0.26	-
NH_4-N	12,500	3700	6	240	6	-
NO_3-N	<3.4	15.2	2400	3.2	5.7	-
Ca	18,000	24,000	21,000	2000	8700	-
Mg	10,900	2000	3100	2900	1580	-
P	27,000	8900	13,300	1090	520	-
K	2000	760	10,200	940	1590	-
Na	720	4200	1550	108	300	-
Mn	139	1170	350	240	165	-
As	5	5	11	5	2	20–30
Cd	0.81	0.39	0.51	0.028	<0.10	0.1–10
Cr	21	17,300	19	19	6	600–1500
Cu	240	108	61	128	8	100–1250
Pb	19.9	12.2	66	23	4.8	300
Ni	18	28	8	12	5	60–135
Zn	620	380	300	175	41	300–1500

Note: * The lower limit indicates maximum concentration for class a biosolids (unrestricted use) while the higher limit indicates maximum concentration for class b biosolids, a limit above which biosolids are not suitable for reuse on land [48].

The biosolids and compost were passed through a 12 mm sieve and homogenised for 20 min. Subsamples of the homogenised biosolids and bark fines were collected for further analysis. Six indigenous species with different ecological requirements (Supplementary Material—Table S1) were selected from those commonly grown in nurseries to be used in indigenous plantings along the country. These were collected from the Garner Park nursery (Levin). The species grown were the following: *Veronica salicifolia*

G.Forst [49,50], *Poa cita* Edgar [51–53], *Corokia cheesemanii* Turril [54,55], *Phormium tenax* J.R.Forst & G.Forst [49,56,57], *Griselinia littoralis* Raoul [51], and *Cordyline australis* (G.Forst.) Endl. [56,58].

The plants were exposed to increasing concentrations of biosolids mixed with bark based on dry weight, resulting in five treatments per biosolids. The fresh biosolids were B1 and B2 (both at 0%, 5%, 10%, 15%, 25% biosolid concentration), and the aged biosolids were B3 and PS (both at 0%, 10%, 20%, 30%, 50%).

The plants were potted into 36-well trays with each plant potted into approx. 150 g of bark or bark + biosolids, with one row for each biosolid concentration and one plant type per tray. Six replicates of each plant species were planted in each biosolid/bark ratio, totalling 720 planted seedlings. A control treatment (bark without biosolids or amendment) was added in each tray to account for differences in the time since planting or placement in the greenhouse.

2.2. Plant Monitoring

The trays with plants were evenly spread into two separate greenhouses, one for biosolids B1 and B2 and another for B3 and PS for even irrigation. Average temperatures at the greenhouses during the day and night were 21.4 °C and 9.2 °C (minimum 9.5 °C and maximum 33 °C). Plants were watered twice a day to pot capacity, and the trays were randomsied around the greenhouses twice a week. The plants were grown until they required more irrigation than twice daily. Hence, the experiment ran for four months during the summer period from late 2018 to early 2019. Plant height and overall health was monitored every two weeks (Supplementary Material).

2.3. Plant Sample Collection

At the end of the experiment, all aerial parts of the plants were harvested and dried to determine aerial dry weight and then put into paper bags and dried in an oven at 60 °C until constant weight (4 days). The leaves of *P. cita*, *P. tenax* and *C. australis* were cut with stainless steel scissors into 2 cm fractions to facilitate grinding. As for the dicot species, leaves were separated from the stem and ground in a Breville coffee and spice grinder (model: BCG200BSS) and stored in zip-lock bags until analysis.

2.4. Biosolid, Pond Sludge, and Bark Analysis

One sample of bark and each biosolid and pond sludge was sent to a commercial laboratory for chemical analysis (Hills Laboratories, Hamilton, New Zealand). The moisture content of the samples was determined by drying a subsample at 103 °C until a constant weight was achieved. Air drying of the samples was performed at 35 °C and then sieved down to <2 mm. The electrical conductivity and pH were analysed at 1:2 and 1:5 (*w*:*v*), respectively, on sample:water ratios on air dried samples. The total organic carbon (TOC) was analysed using an elemental analyser after acid pretreatment to remove present carbonates [59]. The total N was determined by catalytic combustion using an elemental analyser. The ammonium and nitrate were determined on fresh samples by extraction with 2 M KCl and analysed using colourimetric and flow injection analysers [60,61]. The dried samples were acid digested using a microwave digester for further determination of the total elements (Ca, Mg, Mn, P, K, Na, As, Cd, Cr, Cu, Ni, Pb, and Zn) present by ICP-MS [62].

2.5. Plant Analysis

Some 0.2000 (±0.0001) g of ground plant material was weighed into 15 mL borosilicate digestion tubes. Five mL of HNO_3 (Analar 67%) was added to the tubes and then digested using an ultraWAVE microwave acid digester (Milestone Srl, Sorisole, Italy) at 220 °C and 110 bar. Each run included quality control samples (certified reference material, NIST 15739, tomato leaves) and blanks. Elemental concentrations were determined using ICP-MS (7500cx, Agilent Technologies, Santa Clara, CA, USA). A LECO CN828 carbon/nitrogen

analyser (LECO, St. Joseph, MI, USA) was used to determine total C and N in the ground plant samples. Before each run, certified reference material was read to assure the quality of the measurements (Orchard leaves LCRM, LECO corporation, St. Joseph, MI, USA). Recoveries ranged from 72 to 115% of the published values.

2.6. Biomass Index (BI)

The plant biomass index (BI) was obtained as the quotient of the dry biomass of the plant grown on each treatment (Bt) divided by the average dry biomass plant grown on control (Bc, bark with no amendment) [63], show in the following equation:

$$BI = Bt/Bc.$$

The biomass index of the control is, by definition, 1. Results for the biomass index have been split into fresh biosolids and aged biosolids, given the similarities in results obtained between biosolids and pond sludge for each category.

2.7. Statistical Analysis

To account for differences in plant age when planting or other covariables, such as location in the greenhouse, results were compared with each control treatment in each tray. The data were analysed with R-studio [64]. A one-way ANOVA was performed to determine significant differences in plant biomass for each species at increasing ratios of biosolids. Residuals were plotted and tested for normality and homoscedasticity assumptions. Data were log transformed when assumptions were not met. Tukey's post hoc test was used where significant differences were found using the package *multcomp* [65]. An independent two-sample t-test was used to compare each individual ratio of biosolids against the control group (no biosolids). The significance level of the results was at $p < 0.05$.

3. Results

3.1. Biosolid Effect on Plant Biomass

Plants grown on fresh biosolids (B1 and B2), which have high concentrations of macronutrients (Table 1), resulted in consistent plant growth using both biosolids at lower to medium rates. The increasing application rates of fresh biosolids led to a significant increase in plant biomass ($p \leq 0.05$) compared to no biosolid application (BI = 1.0) for most species (Figure 1). The biomass of *G. littoralis* and C. *cheesemanii* were significantly lower at the 25% application rate for both fresh biosolids (B1 and B2). At the 25% rate, *P. cita* developed chlorosis in B2, but this did not occur in the other treatments. *V. salicifolia* had the greatest response for increased biomass among the dicot species and *C. australis* in the case of monocot plants. Both species showed a consistent increase in biomass production while increasing the biosolid rate. *P. cita* developed significantly more biomass at increased rates compared to the control samples (BI = 1.0).

The maximum biomass production occurred at rates of 10–25% in B1 and B2 for all plant species (Table 2). An application rate of 10% B1 and B2, equivalent to 75 kg N ha^{-1} and 242 kg N ha^{-1}, respectively, would provide adequate N for all plant species. In both the B1 and B2 treatments at 10%, *C. australis* and *V. salicifolia* showed a 700% increase in biomass compared to the control (BI = 1.0). In contrast, *G. littoralis* increased its biomass by just 50%.

In the aged treatments (B3 and PS, Figure 2), plant response varied, where some plants had no significant growth response; however, at rates between 20% and 30% of B3, the biomass decreased. *P. tenax* and *P. cita* did not respond to B3 or PS. For *P. tenax*, plant biomass was consistently lower than the control in PS. *G. littoralis* had no response in the PS treatment; however, at rates > 20% B3, it decreased the biomass. *C. cheesemanii* and *C. australis* seemed to have similar responses to both biosolids, having a significant increase at higher ratios. *V. salicifolia* produced significantly more biomass (67%) in B3 compared to PS. However, the leaves of this species developed chlorosis during the trial in PS at the highest rate (50%) [47]. Overall, plants in B3 were not negatively affected at the highest

rates, while PS was phytotoxic at high rates (Table 2). An optimal application rate of 30% of aged biosolids (B3) or PS was equivalent to 121 kg N ha^{-1} and 40 kg N ha^{-1}, respectively.

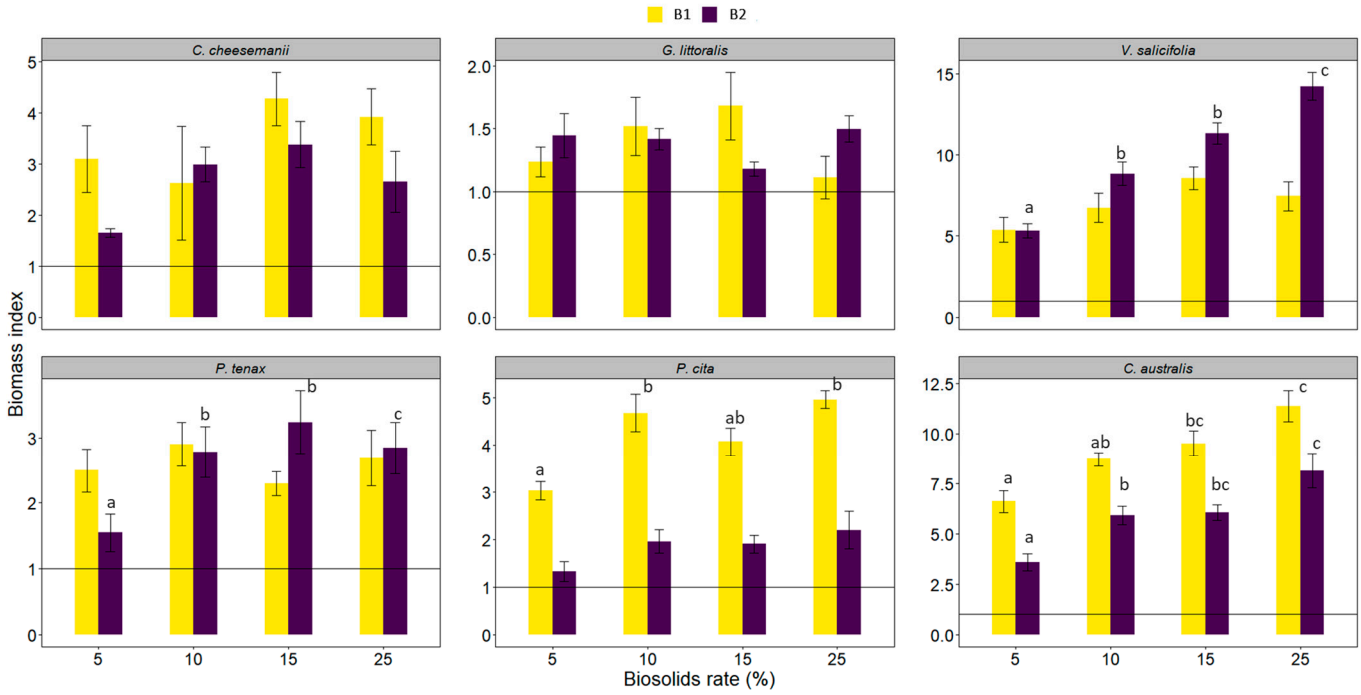

Figure 1. Biomass index of species versus increasing rates of biosolids from biosolid 1 and 2 (B1 and B2). Results show the average and standard error of the mean. The biomass index of control in dicated on the Y axis (y = 1). Letters over bars represent significant differences among the ratios for each treatment (n = 6). Note: The *Y*-axes are in different scales to visualise data patterns.

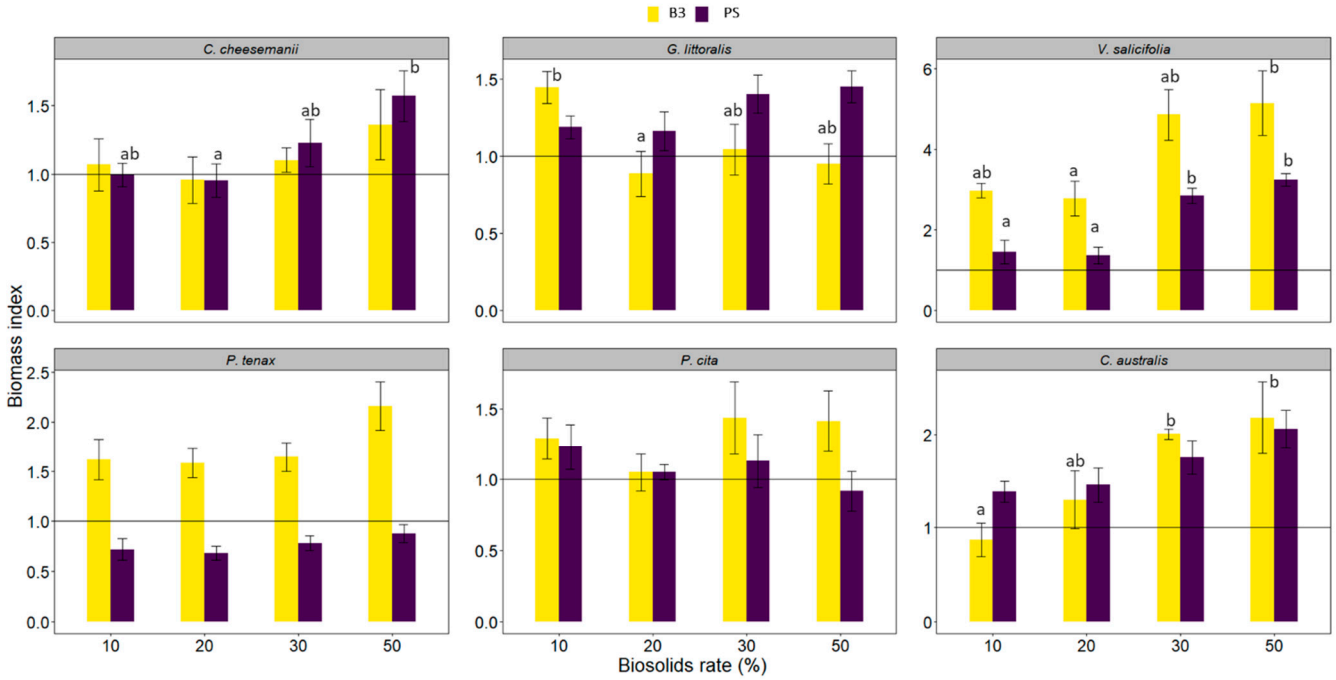

Figure 2. Biomass index of species versus increasing rates of biosolids from biosolid 3 and pond sludge (B3 and PS). Results show the average and standard error of the mean. The biomass index of control in dicated on the Y axis (y = 1). Letters over bars represent significant differences among the ratios for each treatment (n = 6). Note: The *Y*-axes are in different scales to visualise data patterns.

Table 2. Concentration of biosolids (%) that provided the highest biomass.

Plant sp.	B1 (%)	B2 (%)	B3 (%)	PS (%)
C. cheesemanii	15	15	30	30
V. salicifolia	15	25	30	50 *
G. littoralis	15	10	30	30
P. tenax	10	15	50	50
C. australis	15	25	50	50
P. cita	25	25 *	30	30

Note: * Treatments that produced chlorosis in *V. salicifolia* and *P. cita*.

3.2. Plant Nutrition and Trace Elements at the Optimal Biomass Production of Biosolid Addition

The concentration of macro elements in the leaves of the six indigenous species increased significantly for most plants, except for *P. cita* which had a negligible response among all the species and across all the biosolids/pond sludge. Foliar concentrations of N, P, and S responded similarly to biosolid addition, increasing significantly by 360%, 170%, and 880%, respectively (Table 3). In the case of K, even though there was a slight increase in concentration for some species, the opposite was observed in most plants grown under B1 (Table 3). Other major elements such as Ca and Mg increased greatly for some species and, mainly, when B1 was applied (Table S2). The total N and P increased significantly while using B2 biosolid at the optimal rate. However, most of the other elements were not significantly different from the unamended samples.

Table 3. Elemental concentration of macro elements in mg kg^{-1} (dw) (unless otherwise indicated) in control plants versus elemental concentration at the optimal ratio for biosolids B1-PS. Values represent the mean, and values in brackets represent the standard error (n = 6). Significant differences between control and treatment are indicated with an asterisk (*); *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

	N (%)		P		K		S	
	Control	Optimal	Control	Optimal	Control	Optimal	Control	Optimal
Biosolids 1								
C. australis	0.32 (0.05)	1.48 (0.05) ***	1727 (169)	3588 (203) *	9429 (150)	6391 (288) ***	439 (9)	1048 (36) ***
C. cheesemanii	0.55 (0.04)	1.48 (0.04) ***	1272 (282)	1811 (153)	12,893 (554)	10,017 (143) *	131 (10)	1293 (167)
G. littoralis	0.49 (0.04)	1.19 (0.03) ***	1656 (60)	4107 (260) **	14,677 (1462)	11,235 (359)	1022 (102)	2121 (109) **
P. cita	0.31 (0.09)	0.3 (0.03)	1802 (246)	1724 (117)	7437 (610)	9072 (250) *	1242 (168)	1251 (50)
P. tenax	0.50 (0.04)	1.48 (0.09) ***	1658 (29)	4129 (423) **	9829 (574)	9393 (641)	733 (53)	1951 (105) ***
V. salicifolia	0.37 (0.01)	1.55 (0.22) *	1692 (84)	3299 (303) **	14,454 (1098)	11,288 (1185)	1070 (121)	3024 (711) *
Biosolids 2								
C. australis	0.20 (0.12)	0.79 (0.03) *	1301 (133)	2876 (153) ***	6385 (903)	11,777 (552)	288 (12)	619 (30)
C. cheesemanii	0.55 (0.07)	1.4 (0.08) ***	1005 (121)	1843 (83) *	12,047 (799)	12,183 (287)	593 (34)	2011 (420)
G. littoralis	0.35 (0.05)	0.71 (0.05) ***	1484 (98)	2637 (184) ***	14,807 (1227)	17,780 (404)	912 (72)	1823 (156)
P. cita	0.23 (0.02)	0.5 (0.05) **	1026 (43)	2142 (190) **	6439 (347)	10,088 (750) **	934 (43)	1533 (25)
P. tenax	0.20 (0.01)	1.01 (0.05) ***	1267 (79)	3431 (256) ***	7567 (343)	13,554 (776) *	563 (35)	1648 (183)
V. salicifolia	0.49 (0.06)	0.73 (0.04) **	1918 (359)	1904 (100)	18,172 (1097)	15,463 (1680)	1356 (188)	2041 (133)
Biosolids 3								
C. australis	0.21 (0.04)	0.51 (0.08) *	1635 (72)	2794 (74) ***	7889 (990)	11,943 (584) *	513 (82)	483 (26)
C. cheesemanii	0.55 (0.03)	0.77 (0.06) **	856 (46)	935 (135)	9676 (575)	10,171 (643)	656 (33)	912 (147)
G. littoralis	0.67 (0.06)	0.72 (0.05)	1492 (175)	1884 (65) *	10,867 (1448)	16,490 (579) **	1292 (119)	1294 (52)
P. cita	0.37 (0.03)	0.38 (0.02)	1334 (109)	1527 (69)	8936 (775)	10,469 (444)	938 (31)	1102 (68) *
P. tenax	0.30 (0.04)	0.57 (0.05) **	1491 (105)	3019 (269) **	8179 (98)	16,012 (946) ***	688 (6)	904 (25) ***
V. salicifolia	0.38 (0.02)	0.54 (0.07)	1473 (54)	2323 (114) ***	9328 (313)	17,480 (1093) ***	915 (105)	1703 (158) **
Pond sludge								
C. australis	0.15 (0.04)	0.38 (0.04) *	2184 (49)	1733 (115)	9639 (582)	9929 (1115)	403 (53)	410 (8)
C. cheesemanii	0.58 (0.08)	0.82 (0.04)	1319 (178)	1645 (197) *	12,602 (1210)	14,171 (296)	540 (21)	952 (16) ***
G. littoralis	0.35 (0.01)	0.39 (0.01) ***	1323 (67)	1796 (133) *	11,495 (768)	15,616 (1860)	932 (15)	2056 (93) ***
P. cita	0.28 (0.02)	0.27 (0.01)	1255 (115)	1332 (52)	8279 (738)	8621 (211)	815 (62)	891 (32)
P. tenax	0.20 (0.03)	0.19 (0.04)	1777 (84)	2374 (167) *	10,345 (331)	13,801 (790) **	628 (23)	866 (112)
V. salicifolia	0.38 (0.03)	0.54 (0.04) **	1801 (176)	1957 (142)	11,763 (1303)	13,655 (1146)	817 (87)	1774 (154) ***

The leaf concentration of trace elements, mostly Cd, Cr and Zn, in indigenous NZ plants varied from one species to another (Table 4). In the case of *V. salicifolia*, Cr significantly decreased by 30% when using B1 and B2. For Cu, slight changes were observed in some cases. Other trace elements are listed in the Supplementary Data (Table S2). The concentration of these was mostly unaffected by biosolid addition at selected rates.

Table 4. Elemental concentration of trace elements in mg kg^{-1} (dw) in control plants versus elemental concentration at the optimal ratio for biosolids B1-PS. Values represent the mean, and values in brackets represent the standard error (n = 6). Significant differences between control and treatment are indicated with an asterisk (*); *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

	Cr		Cu		Ni		Zn	
	Control	**Optimal**	**Control**	**Optimal**	**Control**	**Optimal**	**Control**	**Optimal**
				Biosolids 1				
C. australis	1.1 (0.14)	1.27 (0.101)	10.9 (0.72)	20.7 (1.96)	0.26 (0.04)	0.73 (0.046) ***	50.9 (6.43)	119 (7.32) ***
C. cheesemanii	0.26 (0.05)	0.32 (0.036)	14.1 (1.63)	9.9 (1.43) *	0.24 (0.1)	0.32 (0.089)	53.8 (4.86)	70.3 (7.56)
G. littoralis	0.25 (0.06)	0.21 (0.035)	22.6 (2.41)	29 (1.92)	0.28 (0.04)	0.41 (0.045)	97.1 (7.4)	156 (14.1) **
P. cita	1.45 (0.35)	1.77 (0.134)	36.7 (9.93)	35.2 (3.48)	0.82 (0.15)	1.02 (0.079)	37.0 (5.29)	24.3 (1.26) *
P. tenax	0.68 (0.07)	0.81 (0.065)	11.5 (0.95)	14.8 (0.53) *	0.4 (0.05)	0.31 (0.06)	80.9 (6.21)	106 (6.18) *
V. salicifolia	10.1 (1.16)	3.4 (0.366) ***	18.8 (0.58)	18.9 (0.54)	0.3 (0.01)	0.35 (0.029)	29.9 (1.78)	61.2 (6.32) *
				Biosolids 2				
C. australis	1.38 (0.2)	3.36 (0.958)	17.9 (4.74)	21.7 (2.25)	0.08 (0.04)	0.42 (0.025)	60.6 (0.38)	76.4 (5.9) *
C. cheesemanii	6.91 (4.08)	3.66 (0.767)	22.6 (3)	14.2 (1.23) *	0.25 (0.12)	0.36 (0.026)	62.9 (19.7)	48.1 (2.34)
G. littoralis	0.49 (0.09)	1.68 (0.441) *	22.6 (1.64)	21.5 (1.5)	0.2 (0.02)	0.54 (0.057) ***	124 (18.5)	99.5 (10.4)
P. cita	3.6 (0.37)	8.1 (3.12)	25.9 (3.28)	25.9 (4.21)	1.3 (0.13)	1.58 (0.301)	28.7 (2.29)	28.8 (3.79)
P. tenax	3.65 (0.23)	7.15 (1.748)	18.5 (1.03)	19.4 (0.99)	0.45 (0.04)	0.73 (0.051) **	86.0 (3.13)	87 (3.23)
V. salicifolia	5.48 (0.26)	2.35 (0.242) ***	21.5 (1.76)	18.1 (0.89)	0.3 (0.02)	0.32 (0.018)	38.5 (2.76)	34 (4.09)
				Biosolids 3				
C. australis	0.42 (0.02)	0.468 (0.169)	13.6 (1.02)	15.5 (3.24)	0.06 (0.01)	0.248 (0.072)	51.8 (4.71)	53 (3.01)
C. cheesemanii	1.81 (0.51)	0.441 (0.092) *	13.4 (1.6)	12.7 (0.99)	0.77 (0.26)	0.136 (0.02)	48.9 (3.99)	46.7 (9.63)
G. littoralis	0.36 (0.02)	0.188 (0.029) **	22.6 (1.89)	23.7 (0.92)	0.34 (0.1)	0.304 (0.06)	119 (14.1)	126 (15.9)
P. cita	2.78 (0.44)	4.21 (0.78)	19.4 (3.31)	13.3 (2.21)	1.41 (0.21)	1.95 (0.301)	28.7 (2.66)	26.5 (4.06)
P. tenax	0.79 (0.06)	0.513 (0.039) **	20.6 (2.77)	17.2 (1.16)	0.49 (0.04)	0.291 (0.023) **	69.1 (3.38)	59.8 (4.05)
V. salicifolia	0.93 (0.12)	0.478 (0.077) **	21.5 (1.91)	20.7 (3.52)	0.27 (0.02)	0.107 (0.011) ***	41.9 (4.31)	27.6 (3.23)
				Pond sludge				
C. australis	0.42 (0.04)	0.427 (0.081)	12.4 (0.97)	11 (0.79)	0.22 (0.01)	0.25 (0.04)	50.1 (1.21)	57.8 (5.06)
C. cheesemanii	0.49 (0.05)	0.551 (0.045)	16.6 (1.71)	28.3 (2.94) **	0.34 (0.07)	0.469 (0.051)	60.5 (3.82)	67.1 (2.77)
G. littoralis	0.36 (0.09)	0.33 (0.057)	16.9 (1.18)	14.8 (0.83)	0.15 (0.01)	0.324 (0.068) *	121 (6.78)	124 (3.71)
P. cita	4.7 (1.57)	3.88 (0.408)	23.2 (2.28)	24.8 (1.92)	2.23 (0.7)	1.85 (0.141)	29 (3.7)	27.2 (0.67)
P. tenax	1.21 (0.14)	1.15 (0.211)	14.3 (0.891)	22.2 (3.35) *	0.23 (0.07)	0.604 (0.082) *	100 (12.8)	104 (15.5)
V. salicifolia	0.54 (0.07)	0.325 (0.039) *	31.5 (4.46)	28.7 (3.12)	0.21 (0.03)	0.308 (0.03) *	39.7 (4.58)	59 (6.21) *

4. Discussion

The increased growth of *V. salicifolia*, *P. tenax*, *G. litteralis*, and *C. australis* with the addition of all three biosolids and pond sludge is consistent with previous research [39,66–68], following the addition of biosolids at 10% *w*/*w* in degraded soils [39,66], irrigated with treated municipal wastewater [67], and under nitrogen loading in the form of urea [68].

For the other species, *C. cheesemanii* and *P. cita*, there is no record of studies using organic amendments with these plants, and there is a lack of knowledge about their nutrient requirements. Their non-significant response to the amendments may be due to the plants being naturally adapted to low fertility environments [69,70]. Other indigenous NZ plants that did not respond to biosolids in previous studies [39] include *Phormium cookianum Ozothamnus leptophyllus*, *Coprosma acerosa*, and *V. salicifolia*, which had no significant biomass increase when biosolids were incorporated into two low-fertility soils, an Orthic brown soil and sand [39].

The aerial biomass (total dry weight) differed between rates of biosolid application and type of biosolids. While at higher rates, generally, more biomass was produced in some species, this led to chlorosis or reduced growth. *V. salicifolia* and *P. cita* developed

chlorosis at rates of 50 and 25% of PS and B2, respectively (Table 2). At the application rate (25% of B1 and B2), there was a significant reduction in the biomass of *C. cheesemanii* and *G. littoralis*. This is likely due to the high concentration of trace elements present in both biosolids, in which B1, Zn, and Cu exceed the current biosolids guidelines for land application [48]. For B2 biosolids, in addition to Zn and Cu being above the NZ guidelines values, the high salinity ([Na] = 4200 mg kg^{-1}; 619 mS cm^{-1}) could have contributed to a decrease in plant biomass for both plants, as previously mentioned by other author [41]. Salinity can reduce plant growth by reducing the ability of plants to take up water [71]. Salinity has been shown to be a factor reducing growth in soils amended with biosolids and composts [72].

The optimal application rate for all plant species for fresh (B1 and B2) and aged (B3 and PS) biosolids was 10% and 30%, respectively. At this optimal rate, the plants can produce significantly higher biomass compared with no biosolid addition (control, only bark) and without signs of chlorosis and/or hindered growth, at least in their early stages.

Chlorosis is a general response of plants to stress [73] and can occur from elevated concentrations in biosolids, including Cu or Zn [73,74], as well as from deficiencies of essential macronutrients and micronutrients such as N, Mn, Fe, Cu, or Zn [74,75]. The indigenous species that showed a positive response could potentially be established for ecological restoration on biosolid-treated soil; however, further research needs to be conducted to better assess their potential in field conditions, particularly their interaction with weeds.

Biosolid addition can result in a paradoxical decrease in some nutrient concentrations, even though these elements occur at high concentrations in the biosolids. If the increase in growth of the plant exceeds the increase in the rate of uptake, then the nutrient can be "diluted by growth" [76].

The nutrient concentrations in most of the species tested (Table 3), fell within the range of previously reported concentrations for plants growing in their natural environment [51]. However, in some cases, nutrient concentrations exceeded other reported concentrations. While this may be the result of luxury uptake [68], it may also indicate excessive uptake resulting in plant stress.

The macronutrients N, P, K, and S were significantly higher on the leaves of plants growing at the selected optimal rates of biosolid application compared with controls, except for K under B1 biosolid, in which case it was lower in most species, except for *P. cita* and *P. tenax*, probably due to different nutrient uptake mechanisms of these species or a dilution effect due to increased biomass [76]. Biosolid 1 had elevated concentrations of NH_4^+, which could have induced a K^+ decrease due to ion competition in the root uptake as monovalent cations with similar hydrated atomic radius [75,77]. Nevertheless, we did not observe K deficiency signs (less leaf area), since the concentrations of K were similar to those that support adequate plant growth (10,000–50,000 mg kg^{-1}) [75].

The foliar concentrations of other macronutrients, such as Ca and Mg, were dependent on both the types of biosolids and the plant species. In the case of B1 (Table S2), Ca and Mg concentrations were significantly higher on *C. australis*, *P. tenax*, and *V. salicifolia* compared to the control, meaning these may be limiting elements. Similar findings were reported by other authors [41,42] measuring *L. scoparium* leaves grown on biosolids incorporated into degraded, low fertility soils.

For trace elements, foliar Zn was significantly higher (30–135% increase) in the plants growing in biosolid B1 than in the control. The responses of foliar Zn varied between plant species and biosolid type. B2, B3, and PS had lower Zn concentrations than B1. Such differences can be attributed to both the substrate chemistry and the plant physiology [66] and how much exchangeable Zn was available for each substrate (not tested). In all cases, the concentrations at optimal rates were below threshold values for phytotoxicity and animal tolerance levels [75,78] (<100–300 mg kg^{-1}; <300–500 mg kg^{-1}, respectively); this indicates that Zn is unlikely to cause food chain toxicity if plants were used in the field for ecological restoration. Non-essential trace elements (As, Cd, and Pb) in leaves were found below phytotoxic ranges for plant growth (3–10 mg As kg^{-1}, <0.280 mg Cd kg^{-1},

and <5 mg Pb kg^{-1} [74,78]. The occasional monitoring of Cd concentrations could ensure food chain protection from this toxic element [74]. Although some trace elements were present in high concentrations in most of the biosolids (Cr, Cu, and Zn), previous research has shown that trace elements contained in biosolids are less available for soil and plant systems; hence, they are less likely to enter the food chain [79].

Previous NZ studies on native vegetation interacting with biosolids (stockpiled biosolids and/or mixed with sawdust) showed similar trace element concentrations in plant foliage to those presented in this work, especially Zn and Cu [39,40,42]. Although these concentrations generally increase in the leaves of plants exposed to biosolids, the foliar concentration is mostly below phytotoxic values. The response, however, is highly variable depending on the species physiology, along with the chemistry of the biosolids and substrate/soil used. As an example, Dickinson et al. [66] found no significant effect on trace elements and nutrients after biosolid addition in the foliage of *G. littoralis* and *P. tenax*. Overseas studies have reported an improvement in the nutritional status of species on contaminated soils amended with biosolids while reducing the transfer of TE to the aerial portions of the plants [28,80,81].

Whereas this study used bark as a substrate, which is commonly used in nurseries, other materials can be used including sand, pumice, or perlite [82,83], and their use with biosolids needs to be addressed. Sand is mostly used to increase the bulk density of the media and can contain a high salt content [82], whereas pumice and perlite are used to improve water retention and decrease the bulk density of the mixtures. Depending on the source, these amendments can have variable pH and cation exchange capacity (CEC), affecting the chemistry of the final mixture [82].

The diverse plant response towards biosolid mixtures, especially at higher rates, suggests that land application of biosolids could lead to an increase in weed or exotic species growth, and, potentially, indigenous species may perform similarly or worse than exotic species [84]. Additionally, biosolid incorporation might alter soil microbial communities either positively or negatively [85]. This strongly depends on the biosolid composition (pH and trace elements particularly), application types/rates, existing microorganisms, and soil type [86].

The beneficial use of biosolids in nurseries can have positive economic outcomes. Using NZ as a case study, where ca. 40,000,000 seedlings yr^{-1} [87] are grown in 0.5 L pots, the reuse in nurseries would result in the consumption of 3000 tonnes of biosolids (at 10% (w/w) application rate), representing 3% of the annual biosolid production in NZ [6]. Landfilling the equivalent amount of biosolids would cost NZD ~1 million per year [88]. In addition, using biosolids would reduce fertiliser consumption by 100 tonnes (e.g., Osmocote) at a value of NZD ~1 million [87,89]. This option for reusing biosolids would save about NZD 2 million per year from landfilling costs and fertiliser usage.

5. Conclusions

Contrasting biosolids mixed with bark at rates of 0–50% produced distinct effects on indigenous NZ plants in terms of biomass production and plant chemistry. While two species (*V. salicifolia* and *C. australis*) benefited from consistent growth and enhanced nutrient status on most biosolids, others (*P. cita* and *G. littoralis*) had no response to these amendments. Our results indicated that the selection of an appropriate rate of 10–30% on a range of biosolids can accelerate the growth of indigenous species on nurseries without increasing trace element concentrations to phytotoxic ranges that could facilitate contaminant entry into the food chain. In the case of using the plants in this research into field trials, the appropriate selection of species is encouraged, as well as prior biosolid and substrate characterisation to apply appropriate amendments rates. The low organic matter, plant nutrients, low pH, and the comparatively high concentration of trace elements of the pond sludge made this an unsuitable substrate for growing indigenous seedlings in nurseries, unless it is further blended with other nutrient-rich material.

While our research demonstrates that biosolids can be effectively used to raise indigenous plants in nursery conditions, future research should delineate the long-term performance of plants raised with biosolids compared to those raised in other growth media.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16091226/s1 Table S1: Ecological requirement by indigenous species used in the greenhouse experiment [49–58]; Table S2: Elemental concentration of macro/micro elements in mg kg^{-1} (dw) in control plants versus elemental concentration at the optimal ratio for biosolids B1-PS; Figure S1: plant height during the experiment using B1. (A) *V. salicifolia*, (B) *C. cheesemanii*, (C) *G. littoralis*, (D) *P. tenax*, (E) *C. australis*, (F) *P. cita*; Figure S2: plant height during the experiment using B2. (A) *V. salicifolia*, (B) *C. cheesemanii*, (C) *G. littoralis*, (D) *P. tenax*, (E) *C. australis*, (F) *P. cita*; Figure S3: plant height during the experiment using B3. (A) *V. salicifolia*, (B) *C. cheesemanii*, (C) *G. littoralis*, (D) *P. tenax*, (E) *C. australis*, (F) *P. cita*; Figure S4: plant height during the experiment using PS. (A) *V. salicifolia*, (B) *C. cheesemanii*, (C) *G. littoralis*, (D) *P. tenax*, (E) *C. australis*, (F) *P. cita*; Figure S5: Plants growing in different treatments using B1 at the end of the experiment; Figure S6: Plants growing in different treatments from B2 at the end of the experiment; Figure S7: Plants growing in different treatments using B3 at the end of the experiment; Figure S8: Plants growing in different treatments using PS at the end of the experiment.

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