Using native riparian barriers to reduce *Giardia* in agricultural runoff to freshwater ecosystems

Cynthia L. Winkworth, Christoph D. Matthaei and Colin R. Townsend

ABSTRACT

Waterway degradation in agricultural settings is caused by direct and diffuse sources of pollution. Waterway fencing focuses on reducing direct faecal contamination, but the extent to which it reduces overland surface runoff of pathogens is unknown. This study evaluated the potential of four riparian treatments to reduce *Giardia* in saturation excess surface runoff entering the waterway. Treatment 1 comprised exotic pasture grass and weeds that regenerated from bare soil between the fence and the waterway in the absence of cattle grazing and was compared with three others comprising monocultural plantings of New Zealand native grassland plants. Runoff experiments involving *Giardia* were performed after planting, both prior to and following the summer growing season. *Giardia* was not detected from any plot prior to cyst addition. In spring the native '*C. secta*', '*A. lessoniana*' and '*C. richardii*' treatments showed significantly greater reductions in *Giardia* in runoff than the 'exotic grasses' treatment, while in autumn the '*C. richardii*' treatment reduced *Giardia* more than the 'exotic grasses/weeds'. A reduction in public health risk should follow from riparian vegetation, whether exotic or native, but with an added benefit in the case of the native tussock grass *C. richardii*, due to the associated lower runoff rate.

Key words | dairying, *Giardia*, riparian barriers, saturation-excess surface water runoff

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INTRODUCTION

The degradation of waterways in agricultural settings has been reported worldwide and is caused by both point (direct) and non-point (diffuse) source pollution containing chemical and biological contaminants (Mawdsley *et al.* 1995; Dosskey 2001). Biological pollutants are of particular concern given the sheer volume produced annually, with more than 272 million tonnes of manure produced by confined cattle alone in the United States (Bradford *et al.* 2006b), and the considerable potential for microbes contained within manure to cause human infection (Mawdsley *et al.* 1995).

In the Otago Province of the South Island of New Zealand, extensive land conversion from sheep/beef farming to dairy farming has occurred during the past 15 years, resulting in considerable increases both in total land area under dairying and animal stocking rates per hectare (Statistics N.Z. 2004). Closely correlated with this increase in dairying has been declining water quality, prompting the regional authority charged with protecting the environment to implement strategies to target causes of farm pollution (ORC 2003b; PCE 2004).

Current strategies in New Zealand echo those employed overseas to minimise waterway degradation by focusing on reducing direct deposition of faecal material into water (Sullivan *et al.* 2007). A fencing initiative has been particularly successful at minimising opportunities for stock to defecate and urinate directly into waterways (ORC 2003a). However, the influence of fencing on the diffuse transport of microbes in surface runoff to waterways is poorly understood.

The protozoan pathogen *Giardia* is an organism reported from bovine hosts worldwide, with high prevalences in newborn calves (10–60%) and low but persistent prevalences in adult cows (5%; O'Handley *et al.* 2000; Olson *et al.* 2004; McAllister *et al.* 2005; Trout *et al.* 2005). Mirroring the global pattern, two New Zealand studies have reported between 31 and 41% prevalence of *Giardia* in dairy calves (Hunt *et al.* 2000; Winkworth *et al.* 2008b). Given such levels of *Giardia* prevalence in dairy cattle, it is not surprising that cysts have been detected in aquatic and terrestrial environments where large cattle populations reside (Brown *et al.* 1998; Thurman *et al.* 1998; Brookes *et al.* 2004). Dairy cattle in many parts of the world predominantly harbour a livestock-specific *Giardia duodenalis* genotype, but such strains have yet to be detected in New Zealand dairy cattle (Hunt *et al.* 2000; Learmonth *et al.* 2003; Winkworth *et al.* 2008a). Rather, and of particular concern, the *G. duodenalis* genotypes identified from New Zealand dairy cattle are identical to those associated with human infections in the same geographical region (Winkworth *et al.* 2008a). Given these epidemiological findings, it is important to develop mitigation measures to reduce *Giardia* movement in runoff.

Methods to reduce diffuse pollution often involve the use of vegetation to increase infiltration and thus reduce contaminants reaching waterways (Fiener & Auerswald 2003; Atwill *et al.* 2006). These riparian barriers are either purposely planted or develop over time by ecological succession on land removed from grazing (Abu-Zreig *et al.* 2004; Hickey & Doran 2004; Mankin *et al.* 2007). The waterway fencing initiative in Otago has created large tracts of ungrazed riparian areas (of exotic grasses and weeds) across farmland. However, we do not know whether exotic riparian plants act to reduce *Giardia* concentrations in surface runoff, nor whether plantings of native species would be comparatively more effective. Winkworth *et al.* (2008c) reported an immediate reduction in *Giardia* reaching a waterway when a recently planted treatment (of the native sedge *Carex secta*; Boott) was compared to a bare soil treatment. Soil disturbance, as a result of planting, probably contributed to the small but significant decline in *Giardia* in surface runoff by decreasing water velocity and promoting surface roughness and infiltration (Fiener & Auerswald 2003; Hsieh & Bolton 2007). In the current study, we compare the capacities of four riparian treatments to reduce *Giardia* runoff to waterways. Treatment 1, which consisted of exotic grasses and weeds that developed in grazer-free riparian strips, was compared with purposely-created monocultural riparian barriers of three New Zealand native grassland species that are often recommended in waterway planting schemes to reduce non-point pollution. The experiment was conducted in spring, soon after planting, and again after a summer growing season.

METHODS

Field site

Preparation of the field site was that described previously by Winkworth *et al.* (2008c). Following removal of the pastoral vegetation, twenty-eight individual plots, each 1 m wide, were created perpendicular to the waterway by inserting stainless steel sheets (1.3 mm thick) lengthwise along each of the 2.5 m long sides to a vertical depth of approximately 15 cm, or to the base of the A horizon. On average, plot slope towards the waterway edge from the top of the plot was 5°.

The twenty-eight plots were randomly assigned to four treatments (seven plots per treatment) and prepared as follows. Seven of the twenty-eight riparian plots were designated as the 'exotic grasses/weed' treatment and did not undergo further manipulation; species in this treatment, consisting predominantly of *Ranunculus repens* (L.), *Holcus lanatus* (L.) and *Trifolium repens* (L.), either germinated from the seed bank or were fast colonisers of the bare soil. The twenty-one riparian plots comprising the other three treatments were planted with either *Anemanthele lessoniana* (Steud.), *Carex secta* or *Cortaderia richardii* (Endl.) in a 2-1-2-1-2 configuration lengthwise down the plot (Figures 1 and 2). At the time of planting, *A. lessoniana*, *C. secta* and *C. richardii* were transferred directly from 2.8 liter (L) potting bags into similarly sized holes. *A. lessoniana* and *C. richardii* are both true tussock grasses that under moist conditions typically grow to heights of up to 1.5 and 3 m, respectively (Cave & Paddison 2005). *Carex secta* is a sedge that grows up to one metre high and is commonly found beside water and in
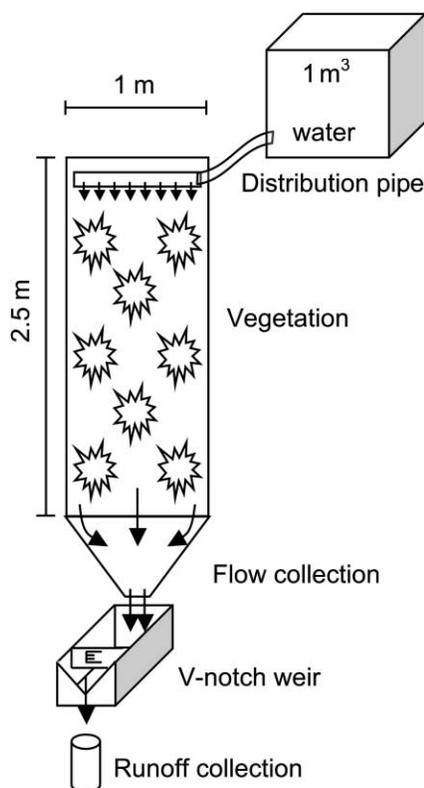
Figure 1 | Diagram of field-based experimental setup showing the plot arrangement, water supply and runoff collection.

swampy conditions (Cave & Paddison 2005). All three taxa are native, endemic New Zealand species. Planting occurred in May 2006, two months after herbicide application to remove existing pastoral vegetation. No plot maintenance occurred before the two *Giardia* runoff experiments, which were performed in spring (22 October to 7 November 2006) and autumn (14 March to 9 April 2007).

All *A. lessoniana* and *C. secta* plants survived to the end of the study. Eight of the fifty-six *C. richardii* plants from three plots died before the spring runoff experiment. On one plot two plants had died away completely, while three showed no evidence of green vegetation. This plot was sacrificed at the conclusion of the spring runoff experiment and its three remaining healthy plants transferred to two other plots where no vegetative evidence could be found for one and two plants, respectively. Thus, the sacrificed plot was only tested in spring. Between the two runoff experiments, one *C. richardii* plant on each of four plots

(one being a transferred plant) had died, as judged from a lack of any green vegetation and a decrease in plant circumference. Nevertheless, these plots were included in the analyses.

Vegetation indices

To provide an index of the development of biomass at ground level, total plant stem circumferences of the native plants were measured for all living individuals on two sampling occasions (22 October 2006 and 9 March 2007). After the summer growing season, estimates were also made of the extent to which the three native grassland treatments had been colonized by non-planted vegetation. First, the percentage of canopy made up of non-planted species was estimated for each replicate in the two metres closest to the waterway using two 1×1 m grids (each comprising one hundred $100 \, cm^2$ sections). Viewed from directly above, the proportion of $100 \, cm^2$ sections (out of a possible one hundred) where the majority of foliage was made up of non-planted species, as opposed to those with a majority of planted species foliage, was recorded as the percentage of non-planted species canopy. Thus, the 'exotic grasses/weed' treatment had 0% representation of planted

Figure 2 | Photographs illustrating the state of the replicate riparian vegetation plots (a) in spring, five months after the plots had been cleared and native species planted and (b) in autumn, after the summer growing season. One replicate is shown for each of the four treatments (in order from left to right): '*Anemanthele lessoniana*', '*Cortaderia richardii*', '*Carex secta*' and 'exotic grasses/weed'.

native taxa. Second, the number of non-planted species that had colonised each replicate of the four treatments was recorded.

Giardia cyst generation

Non-viable cysts were prepared by encysting laboratory-adapted strains of *Giardia* grown in tissue culture following protocols described by Winkworth *et al.* (2008c). The applied spike of *Giardia* contained 7×10^6 cysts suspended in 200 mL of on-site bore water.

Runoff simulation and collection

A ground-level drip irrigation system was used to generate saturation-excess surface runoff across individual plots, as described previously by Winkworth *et al.* (2008c). Water was pumped at a constant rate of 2 L per minute, generating the saturated soil conditions typically observed during late autumn, winter and early spring months in New Zealand (Muirhead *et al.* 2006b). The application of water directly to the plot surface was to create saturation-excess surface runoff broadly comparable to movement down a hillside and was not intended to represent rainfall of a particular magnitude (Collins *et al.* 2004). Plots were deemed saturated when a constant surface runoff rate was measured ten minutes apart at the lower edge of the plot at the outflow collection point.

Two runoff experiments were performed, corresponding to periods when saturation-excess surface runoff can occur; prior to (spring) and following the main summer growing season (autumn) in the Otago region. While saturation-excess surface runoff can also occur during the winter months, an equivalent experiment was not performed during this season as livestock are typically moved to fields cultivated with crops grown specifically for winter grazing (for example, *Brassicaceae* spp.; McDowell *et al.* 2006).

The objective of producing saturated soil conditions and runoff rates that were constant between successive ten minute samplings was achieved in all twenty-eight plots in spring. However, saturated conditions and constant runoff could not be achieved in the autumn runoff experiment for one plot each from the '*A. lessoniana*', '*C. secta*' and '*C. richardii*' treatments despite the application of over

1,000 L of water to each plot. Consequently, these three plots were excluded from the autumn analyses, in addition to the '*C. richardii*' plot sacrificed at the conclusion of the spring sampling owing to the death of five of the eight plants. This left five replicates for '*C. richardii*' and six replicates each for '*C. secta*' and '*A. lessoniana*'. All seven 'exotic grasses/weeds' replicates were tested and analysed in the autumn runoff experiment.

Bromide was used as a non-reactive, nonsorbing, conservative tracer for both the spring and autumn experiments, at final concentrations of 2.4 g/L and 1.8 g/L, respectively (Kouznetsov *et al.* 2007). Water sourced from the on-site bore exhibited a bromide concentration of no more than 3 mg/L.

Each plot was manipulated using the protocol described by Winkworth *et al.* (2008c), bar one exception; fifteen one-minute-runoff samples, as opposed to twenty, were collected at the lower edge of the plot. Logistical constraints ruled out manipulating all twenty-eight plots on the same day; thus, one randomly selected replicate of each of the four vegetation treatments was randomly assigned to one of seven blocks, with one block tested per experimental day. The four plots assigned to each block were also tested in a random order during each experimental day. The seven blocks were manipulated within a period of 30 days in the spring experiment and 17 days in the autumn experiment. Runoff samples were stored at 4°C in the dark until processed.

Calculating concentrations

Bromide and cyst analyses followed protocols described by Winkworth *et al.* (2008c). Bromide concentrations for each minute-long runoff sample collected after application of the spike were adjusted by the dilution factor observed during the experiment as follows.

Bromide recovered (g/L) = original mg/L collected

$$\times \text{ runoff volume collected (mL)}$$

To standardise findings between runoff experiments, the proportional recovery of bromide was calculated by dividing the mean concentration recovered (C_B) for each of

the four treatments by the original concentration applied (Co_B) in the spike for individual time points, as well as for the overall totals recovered. Graphs to characterise the physiochemical processes governing transport of the solute (termed breakthrough curves) were generated by plotting the proportional recoveries of bromide (C_B/Co_B) against time.

For cyst enumeration from each 1-min 2 L runoff sample, 250 mL was pelleted using centrifugation (5,000 × g) for 5 min at 4°C, the supernatant was discarded and the pellet resuspended in phosphate-buffered saline. Cyst concentrations were enumerated from 10 μL of the concentrated sample using a MeriFluor kit (Meridian Bioscience Inc., Cincinnati, OH) (Winkworth *et al.* 2008c). As sub-sampling was performed for *Giardia* cyst enumeration, the resulting raw counts were adjusted to represent the concentration of cysts present in each entire runoff sample collected, using the following equation:

$$No.\,cysts/2\,L = (no.\,cysts/well)(concentrate\,volume/10\,\mu L)$$

$$\times (runoff\,volume/250\,mL)$$

Assuming a minimum concentration volume of 2 mL, the detection limit for individual samples was 10^3 cysts per 2 L.

To enable comparisons between total *Giardia* numbers recovered from the four treatments, the mean number of cysts collected over the entire fifteen-minute runoff period was calculated following methods described by Winkworth *et al.* (2008c). Briefly, cysts in each of the fifteen consecutive minute-long samples collected from the same plot were enumerated using the method described above and the resulting means combined to reflect the total number of *Giardia* recovered from that plot over the entire period tested. As with bromide, to standardise findings from the two runoff experiments the proportional recovery of cysts was calculated by dividing the total number of cysts recovered (C_G) from each treatment by the original total number of cysts applied (Co_G; C_G/Co_G). The proportional recoveries of *Giardia* were then plotted against time for the treatments. Thus, the reduction of *Giardia* cyst numbers could be compared between the four different treatments, as well as between the fifteen consecutive time points.

Average relative *Giardia* to bromide ratios for the four treatments were calculated by dividing the *Giardia* recovery proportion (C_G/Co_G) by the bromide recovery proportion (C_B/Co_B), following a previously described protocol (Harvey & Garabedian 1991; Muirhead *et al.* 2006a).

Statistical analysis

All analyses were performed using SPSS version 13 for Mac OS X (SPSS Inc, Chicago, IL, USA). Results from the two experimental periods were analysed separately because the number of treatment replicates differed between the two runoff experiments (twenty-eight plots in spring and twenty-four plots in autumn; see above). After exploratory analysis, data were log^{10}-transformed where necessary to improve normality and homoscedasticity.

Nested three-way, repeated-measures Analysis of Variance (RM-ANOVA) was used to compare the concentration of *Giardia* cysts recovered from individual 'time-point' samples collected over the fifteen-minute runoff period between the four treatments. The within-subjects factor in this ANOVA model was 'time' (1-15), while the between-subjects factors were 'plot type' ('*A. lessoniana*', '*C. secta*', '*C. richardii*' and 'exotic grasses/weed'; fixed factor), 'block' (1-7; random factor without interaction term) and 'subsample' (1-3; nested within 'plot type'). The results for the nested factor sample are presented in Tables 2 and 3 but are not discussed any further because they merely indicate if the values of a given response variable differed between the three 10 μL-subsamples enumerated for each plot at each time-point, and such differences are irrelevant to the research objectives of this study. In all cases where the assumption of data sphericity was violated, the overall results of the within- and between- subjects analyses were corrected using the Greenhouse–Geisser method (Quinn & Keough 2002). If a between-subjects effect was significant in the ANOVAs, pair-wise post-hoc comparisons were conducted. These were Tukey HSD tests, except in cases of persisting heteroscedasticity, where we performed Games–Howell tests, which do not assume equal variances between groups (Quinn & Keough 2002).

Bromide concentrations and *Giardia* to bromide ratios in the fifteen time-point samples were both analysed with two-way RM-ANOVAs (within-subjects factor 'time').

In this model, 'plot type' was the only fixed between-subjects factor (because a single value for each response parameter was determined per experimental plot at each time point), while 'block' was a random between-subjects factor.

For each runoff experiment, total runoff volumes collected from the plots were compared between the four treatments using a two-way ANOVA without an interaction term. Here 'plot type' was the fixed factor (a single runoff value was measured once per experimental plot) and 'block' the random factor.

To assess plant sizes in spring and autumn, a one-way ANOVA was performed in each season to compare stem circumferences (living plants only) between the three native species (*A. lessoniana*, *C. secta* and *C. richardii*). To assess colonisation by non-planted vegetation in autumn, percentage non-planted canopy cover per treatment and total species richness of non-planted taxa were compared between all four plant treatments ('*A. lessoniana*', '*C. secta*', '*C. richardii*' and 'exotic grasses/weed') using another one-way ANOVA.

RESULTS

Vegetation

Average plant circumferences differed significantly on the spring sampling occasion, with *C. richardii* being larger than *C. secta* and *A. lessoniana* (Table 1). By autumn, however, plant circumferences had increased by 183% for *C. secta*, 155% for *A. lessoniana*, and 138% for *C. richardii*, effectively negating the initial circumference differences.

The percentage of canopy coverage by non-planted taxa during the autumn runoff experiment was highest in the 'exotic grasses/weed' treatment (which was completely covered), intermediate in the '*C. richardii*' treatment and lowest in the '*A. lessoniana*' and '*C. secta*' treatments (Table 1). The 'exotic grasses/weed' treatment comprised more non-planted taxa than the '*A. lessoniana*' treatment, with significant differences in plot colonisation by non-planted vegetation detected between the two treatments (Table 1). Nine colonizing plant species were identified in all plots combined (in descending order of frequency of occurrence): *Ranunculus repens* (L.), *Holcus lanatus* (L.), *Trifolium repens* (L.), *Taraxacum officinale* (F.H. Wigg.), *Rumex obtusifolius* (L.), *Dactylis glomerata* (L.), *Cirsium vulgare* (Savi) Ten., *Senecio minimus* (Poir.) and *Cerastium fontanum* (Baumg.). Two further grass species were also detected (on eight and five plots, respectively) but could not be identified to species.

Runoff

The amount of runoff collected (expressed as a percentage of that applied) was significantly lower from the '*C. richardii*' treatment compared to the 'exotic weeds/grasses' treatment

Table 1 | Mean ground level plant circumferences of the three planted vegetation treatments (in spring and autumn) and percentage coverage by non-planted taxa, total species richness of non-planted taxa (both in autumn) and percentage runoff volume collected (in the spring and autumn runoff experiments) from the four vegetation treatments ('*Anemanthele lessoniana*' [AL], '*Cortaderia richardii*' [CR], '*Carex secta*' [CS] and 'exotic grasses/weed' [EW]). Results (*P*-values) of the associated one-way or two-way ANOVAs are also given

|	Vegetation treatment					*P*-values		
	AL	CR	CS	EW	df	Treatment	Block	Post-hoc test rankings
Mean ground level plant circumference –spring; cm (SE)	25.0 (1.1)	31.7 (2.1)	26.7 (0.9)		2	0.01		CR>(CS = AL)
Mean ground level plant circumference –autumn; cm (SE)	38.9 (0.7)	43.9 (5.5)	48.9 (2.1)		2	0.095		
Canopy cover–non-planted taxa in autumn; % (SE)	23.9 (4.7)	67.3 (6.9)	20.6 (2.9)	100 (0)	3	<0.0001		EW>CR> (CS=AL)
Total species richness–non-planted taxa in autumn (SE)	3.3 (0.5)	5.8 (0.9)	3.8 (0.4)	6.0 (0.8)	3	0.02		EW>AL
Runoff collected–spring; % (SE)	68 (6)	65 (0)	75 (7)	83 (7)	4	0.045	0.03	EW>CR
Runoff collected–autumn; % (SE)	44 (11)	46 (4)	53 (6)	69 (8)	3	0.03	0.02	EW>CR
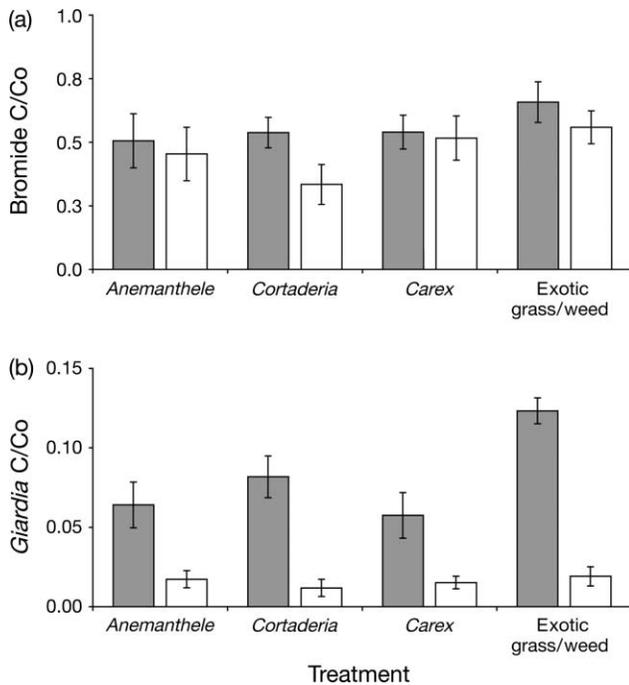
Figure 3 │ Mean breakthrough totals (C/Co; error bars represent one SEM, $n = 5 - 7$) of (a) bromide and (b) *Giardia* cysts collected from the four treatments ('*Anemanthele lessoniana*', '*Cortaderia richardii*', '*Carex secta*' and 'exotic grasses/weed') in spring (grey bars) and autumn (white bars). Note the different scales on the Y axes.

in both spring and autumn (Table 1). Additionally, runoff values were affected significantly by the block factor. On average, lower overall runoff rates were observed in autumn (55% [5 Standard Error of the Mean; SEM]) than in spring (73% [4 SEM]).

Bromide

Overall, bromide recovery from the four vegetation treatments was high, ranging between 76–93% in spring and 54–92% in autumn (Figure 3(a)). Bromide recovery rates were similar across treatments during the spring experiment, while significantly less bromide was recovered from the '*C. richardii*' treatment compared to the 'exotic weeds/grasses' treatment in the autumn (Table 2; between-subjects effects). Further, bromide runoff patterns differed between individual time points, as indicated by significant time effects (in both seasons) and time by treatment interactions in spring (Table 3; within-subjects effects). Significant block effects were found during both seasons.

Enriched bromide concentrations were detected the first minute after spike addition (time 2) in all four treatments in spring (Figure 4). Peak concentrations occurred in the 'exotic grasses/weed' treatment two minutes later (time 4), while in the three native plant treatments the peaks were delayed a further minute (time 5; Figure 4). The general shape of the bromide breakthrough curves in spring for all four treatments was a steep increase to a distinct peak followed by a consistent decline to less than 6% by time 9 (Figure 4).

While the timing of the initial detection of enriched bromide concentrations was the same in autumn as in spring, delays were observed in three of the four treatments for the subsequent bromide peaks. The longest delay was

Table 2 │ Between-subjects results of the repeated-measures ANOVAs comparing bromide and *Giardia* recoveries among the four vegetation treatments ('*Anemanthele lessoniana*' [AL], '*Cortaderia richardii*' [CR], '*Carex secta*' [CS] and 'exotic grasses/weed' [EW]) in the (a) spring and (b) autumn runoff experiments

	Bromide			*Giardia*		
	df	*P*-value	Post-hoc test rankings	df	*P*-value	Post-hoc test rankings
(a) Spring						
Treatment	3	0.38		3	<0.0001	EW>(AL=CR=CS)
Block	6	0.02		6	0.09	
Sample (Treat)				8	0.97	
Error	18			66		
(b) Autumn						
Treatment	3	0.004	EW > CR	3	0.03	EW>CR
Block	6	<0.0001		6	<0.0001	
Sample (Treat)				8	1.00	
Error	14			54		

Table 3 | Within-subjects results (overall effects) of the repeated-measures ANOVAs comparing bromide and *Giardia* recoveries among the four vegetation treatments in the (a) spring and (b) autumn runoff experiments

	Bromide		Giardia	
	df	P-value	df	P-value
(a) Spring				
Time	1.80	<0.0001	1.86	<0.0001
Time*Treatment	5.4	0.003	5.6	<0.0001
Time*Block	10.8	0.27	11.2	0.006
Time*Sample (Treat)			14.9	1.00
Error (Time)	32.4		123	
(b) Autumn				
Time	1.50	<0.0001	4.44	<0.0001
Time*Treatment	4.5	0.096	13.3	<0.0001
Time*Block	9.0	0.40	26.6	<0.0001
Time*Sample (Treat)			35.5	1.00
Error (Time)	21.1		240	

in the 'C. richardii' treatment at six minutes post-spike (time 7; Figure 4(b)), followed by the 'C. secta' and 'exotic grasses/weed' treatments at five minutes post-spike (time 6; Figure 4(c and d)). No delay was observed for the 'A. lessoniana' treatment compared to the spring timings (Figure 4(a)). In general, autumn bromide breakthrough curves were flatter than in spring and took longer to return to less than 5% (Figure 4).

Giardia

Giardia cysts were not detected from any plot prior to addition of the spike during either runoff experiment. The percentage of *Giardia* recovered from the four treatments was higher in spring than autumn, yet overall, the percentage of *Giardia* recovered from the four treatments was low, ranging between 5.8 to 12.4% in spring and 1.2 to 2.1% in autumn. In spring, the 'C. secta', 'A. lessoniana' and 'C. richardii' treatments showed a significantly greater reduction in the proportion of *Giardia* present in runoff than the 'exotic grasses/weed' treatment (Table 2; Figure 3). In autumn, the 'C. richardii' treatment reduced *Giardia* presence in runoff significantly more than the 'exotic weeds/ grasses' treatment (Table 3). Further, *Giardia* recovery values were affected significantly by the block factor in autumn.
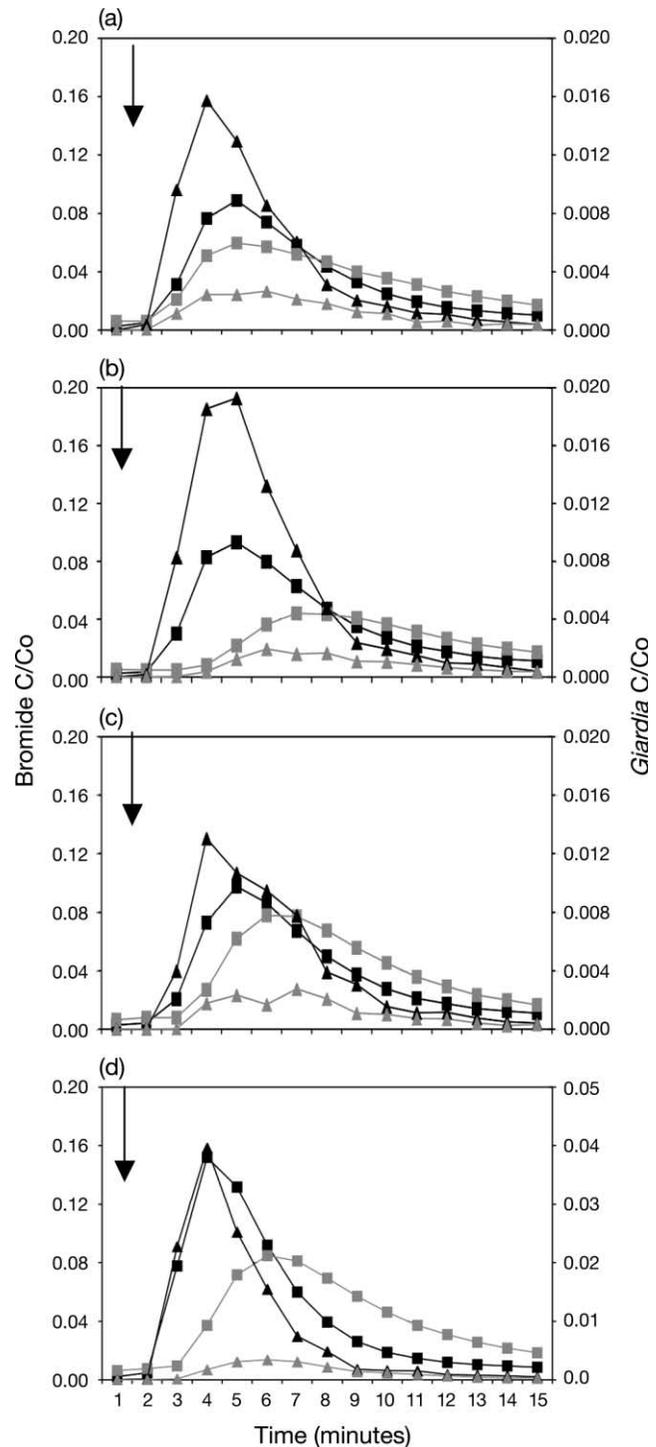

Figure 4 | Mean breakthrough curves for bromide (□) and *Giardia* cysts (△) in surface runoff collected once per minute from the four vegetation treatments (a) 'Anemanthele lessoniana', (b) 'Cortaderia richardii', (c) 'Carex secta' and (d) 'exotic grasses/weed' for the experiment's duration (one minute prior to spike application and fourteen minutes following) in the spring (black lines) and autumn (grey lines). Arrows indicate the time of spike addition. Note the different scales on the two Y axes in (a) (b) and (c) compared to (d).
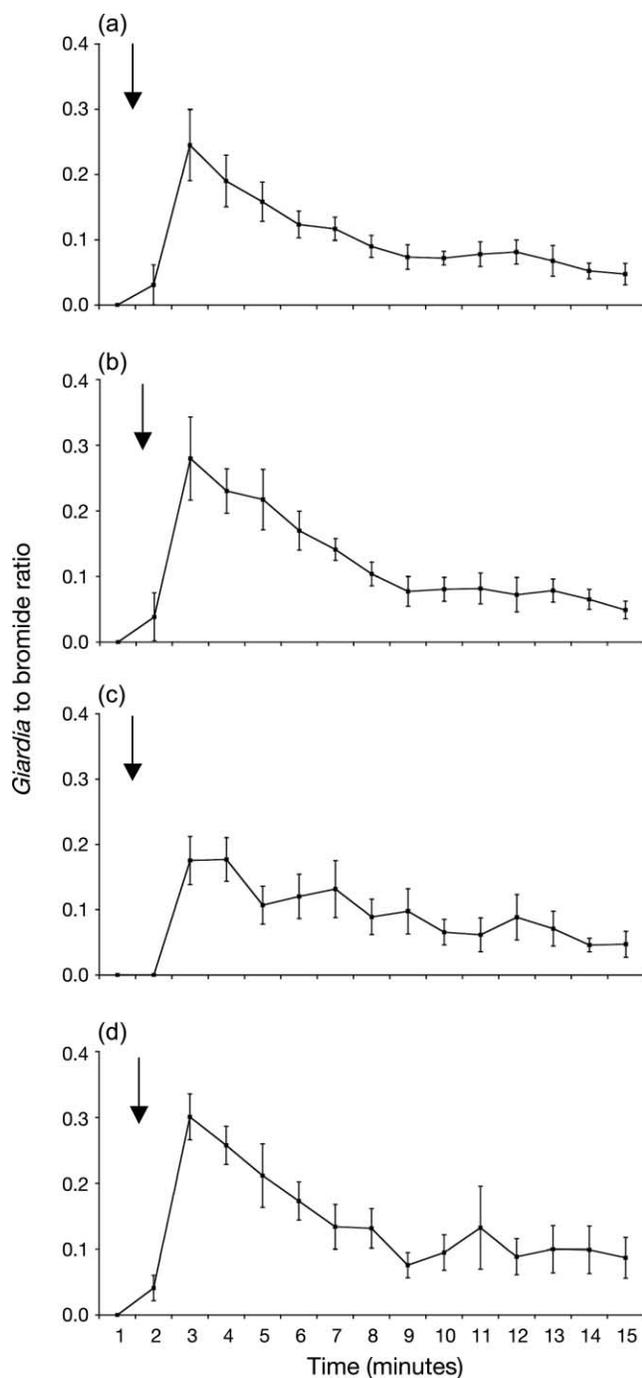
Figure 5 | Relative *Giardia* to bromide ratios ($[C_B/Co_B]/[C_G/Co_G]$; error bars represent one SEM, $n = 7$) calculated from the breakthrough curves of the four vegetation treatments (a) '*Anemanthele lessoniana*', (b) '*Cortaderia richardii*', (c) '*Carex secta*' and (d) 'exotic grasses/weed' over the experiment's duration (one minute prior to spike application and fourteen minutes following) in spring. Arrows indicate the time of spike addition.

The patterns of *Giardia* concentrations changed between individual time points in both runoff experiments, as indicated by several significant within-subjects effects (Table 3). In spring, *Giardia* was first detected during the first minute after spike addition (time 2) in all vegetation treatments except the '*C. secta*' treatment, where it was detected at time 3 (Figure 4). *Giardia* recovery peaked at time 4 in all treatments (Figure 4(a, c and d)) except '*C. richardii*', in which it peaked a minute later at time 5 (Figure 4(b)). In autumn, the timing of initial *Giardia* detection and subsequent *Giardia* peaks were later in all treatments than in spring. *Giardia* was first detected from the '*A. lessoniana*' and 'exotic grasses/weed' treatments two minutes after spike addition (time 3; Figure 4(a and d)), while detection in the '*C. richardii*' and '*C. secta*' treatments was a minute later at time 4 (Figure 4(b and c)). Overall, the *Giardia* peaks were less well defined and flatter in autumn than in spring.

Giardia to bromide ratios

In spring, the *Giardia* to bromide ratios for all four vegetation treatments were similar overall at 0.30 or less ($p = 0.46$; two-way repeated-measures-ANOVA; between-subjects effect) and showed comparable patterns across the fifteen time points, with initial peaks followed by gradual declines (Figure 5; Table 4; no significant time by treatment interactions). Initial detection was delayed by one minute in the '*C. secta*' treatment but all four treatments displayed *Giardia* to bromide peaks two minutes after application of the spike (time 3; Figure 5).

Table 4 | Within-subjects results (overall effects) of the repeated-measures ANOVAs comparing *Giardia* to bromide ratios between the four vegetation treatments in the (a) spring and (b) autumn runoff experiments

	df	*P*-value
(a) Spring		
Time	5.35	<0.0001
Time*treatment	16.04	0.62
Error (time)	128.31	
(b) Autumn		
Time	2.55	<0.0001
Time*treatment	7.65	0.61
Error (time)	50.97	

Autumn *Giardia* to bromide ratios for the four treatments were significantly lower than in spring, with maximum values all less than 0.07 (t-test, $p < 0.001$; $n = 28$ in spring and $n = 24$ in autumn). While overall the patterns were comparable across the four treatments ($p = 0.91$; two-way repeated-measures-ANOVA; between-subjects effect), the timing of the initial peaks and subsequent declines were less well defined than in spring (compare Figure 6 with Figure 5).

DISCUSSION

The principle finding of the present experiment is that in the absence of grazing, riparian barriers (whether purposely planted or not) are capable of reducing *Giardia* in surface runoff. The potentially complicating effects of uneven plant survival in the planted treatments and the prospect of *Giardia* cysts already present on the landscape did not confound the results. For example, no significant differences were detected among runoff volumes and bromide concentrations from the three native treatments, despite mortality or marginal growth of more than 20% of individuals of one of the native grassland species (*C. richardii*). Furthermore, though 5% of cattle excrete roughly 10^6 *Giardia* a day (Heitman *et al.* 2002; Learmonth *et al.* 2003), we did not detect any cysts in runoff collected before application of the *Giardia* spike in either spring or autumn.

Effects soon after riparian planting

In spring, soon after the native species had been planted and when the 'exotic grasses/weed' treatment was still almost bare, significantly more total runoff was recovered from the 'exotic weeds/grasses' treatment than from the '*C. richardii*' treatment. However, this finding was probably the result of different degrees of soil disturbance in the two vegetation treatments, rather than to effects of the plants themselves. While no significant differences in total bromide recovered from the four treatments were observed, a significant time by treatment interaction in bromide recovery was detected across the 15 time points of the runoff experiment. The shape of this interaction indicated that water movement in the three planted treatments was
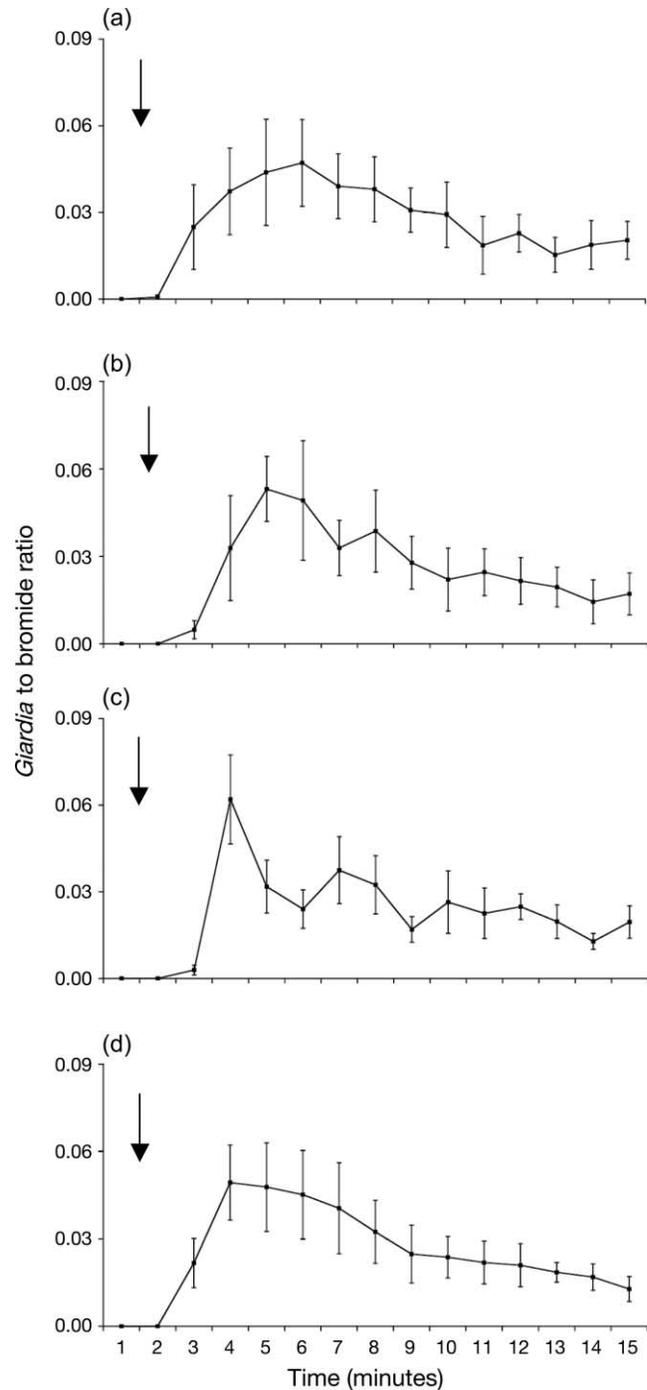

Figure 6 | Relative *Giardia* to bromide ratios ($[C_B/Co_B]/[C_G/Co_G]$; error bars represent one SEM, $n = 5 - 7$) calculated from the breakthrough curves of the four vegetation treatments (a) '*Anemanthele lessoniana*', (b) '*Cortaderia richardii*', (c) '*Carex secta*' and (d) 'exotic grasses/weed' over the experiment's duration (one minute prior to spike application and fourteen minutes following) in autumn. Arrows indicate the time of spike addition.

probably influenced by soil disturbance during planting, compared to minimal disturbance on the 'exotic grasses/ weeds' treatment, with runoff velocity subsequently reduced in the three planted treatments. Owing to the relatively high and comparable levels of bromide recovered from the four treatments, we conclude that the spring sampling was conducted, as intended, with little drainage loss. This finding concurs with previous reports that soil disturbance and the presence of sparsely distributed vegetation can promote surface roughness, decrease water velocity and increase infiltration (Hsieh & Bolton 2007; Miller *et al.* 2007).

Though no vegetation treatment effects were detected for total bromide recovery, significantly more *Giardia* cysts were recovered from the 'exotic weeds/grasses' treatment (87.6% reduction), where very little soil disturbance had occurred, than from all three planted treatments at this early stage in riparian development (92–94% reduction). For comparison, percent reduction in the bare ground treatment in a previous experiment was 81% (Winkworth *et al.* 2008c). Our results agree with a laboratory study on similar pathogens where Trask *et al.* (2004) recovered more *Cryptosporidium* oocysts from runoff traversing bare ground compared with vegetated surfaces at a range of runoff flow rates and slope angles.

Effects after the summer growth season

Substantial changes in vegetation characteristics had occurred between the spring and autumn experiments, with cracks in the soil observed in fields surrounding the experimental plots as a consequence of low rainfall in the interim. Given total surface runoff recovery was generally lower than in the spring experiment (55% cf. 73%), it was likely cracks had also developed on the plots themselves, channelling water away from the surface, and thus reducing or preventing surface runoff. The likelihood of cracked soil in some of the plots by autumn was further supported by our inability to generate saturation-excess surface runoff on three plots (one plot each for '*A. lessoniana*', '*C. secta*' and '*C. richardii*'). The application of over 1,000 L of water to each of these plots was insufficient to generate constant surface runoff, compared with an average of 200 L achieving

this aim in the remaining 24 plots. In spite of the likely presence of cracks in some of the plots increasing between-plot variation in runoff rates, we found significant differences in runoff rates between the 'exotic weeds/grasses' and '*C. richardii*' treatments, with less runoff recovered from the latter.

Overall, the rate of total bromide recovered from the four vegetation treatments following the growing season remained largely comparable with that recovered in the spring, despite flatter fifteen-minute breakthrough curves observed for all four treatments. However, more bromide was recovered in autumn from the 'exotic weeds/grasses' treatment than from the '*C. richardii*' treatment, primarily due to a decrease in bromide recovered from the latter between spring and autumn.

Of particular importance is that the average total concentration of *Giardia* recovered in autumn (98% reduction compared to the contents of the spike) was lower than the concentrations recovered from all three native treatments in spring (93% reduction on average). This difference equates to an average improvement of 79% in the removal of *Giardia* from surface runoff between spring and autumn, indicating increased soil disturbance as a result of the developing vegetation generally led to more efficient *Giardia* reductions. Moreover, the considerable differences in the coverage and richness of exotic colonists in the native riparian buffers made relatively little difference to *Giardia* reduction capabilities. Of the three planted treatments of native grasses, only the '*C. richardii*' treatment removed *Giardia* more efficiently from runoff than the 'exotic grasses/weeds' treatment, which had simply been released from the pressures of grazing and left to regenerate naturally from the seed bank or by fast colonisers. In other words, soil disturbance from the regenerating vegetation on the 'exotic grasses/weeds' treatment was just as effective at channelling runoff into the soil matrix, via increased infiltration, and subsequently retaining *Giardia* in the matrix, as that occurring on two of the planted riparian treatments ('*A. lessoniana*' and '*C. secta*').

Greater rates of infiltration and lower rates of recovery of *Giardia* in runoff were expected for the deeply-rooted grass *C. richardii* compared to the shallow-rooted *A. lessoniana* and *C. secta* (Lee *et al.* 2000). However, all three native plant species had similar effects on water runoff

volumes, bromide and *Giardia* cyst concentrations, ruling out differential root characteristics resulting during the study's duration. It is possible insufficient time had elapsed for significant differences on the measured response parameters to be detected between the three native plant species, as a previous study reported at least three years were necessary for the effects of deep tap roots to become apparent for nutrients and bromide (Dosskey *et al.* 2007). Nevertheless, significant differences were observed between the effects of the 'exotic weeds/grasses' and '*C. richardii*' treatments for all three response parameters, indicating deeper roots may have begun developing during the growing season, channelling surface runoff, bromide and *Giardia* cysts away from the plot surface and deeper into the soil matrix. Consequently following successive growing seasons, additional pathogen reductions may result through an improvement in deep soil infiltration by plant roots.

The mechanism of *Giardia* reduction

Three mechanisms have been reported for the deposition of pathogens in soil: attachment, mechanical filtration and straining. First focusing on attachment, this mechanism involves the deposition and subsequent adherence of pathogens (which act as colloids) to the soil matrix via colloid–colloid interactions (Bradford *et al.* 2006*a*). Such interactions are governed by mechanisms including Lifshitz-van der Waals, electrostatic and acid–base interactions, which in turn depend upon environmental parameters such as soil type, pH and saturation, as well as colloid size, surface charge and degree of hydrophobicity (Ferguson *et al.* 2003; Dai *et al.* 2004). However, a previous study employing similar saturation-excess surface runoff conditions reported that limited *E. coli*-soil interaction occurred and, as such, adhesion to soil (Muirhead *et al.* 2006*b*). Given similar surface runoff rates were employed in the two experiments, it is likely such interaction-limitations were also present in the current study, minimising *Giardia* reduction by way of colloid–colloid attachment.

Turning to the other two mechanisms that govern pathogen deposition, mechanical filtration retains colloids that are physically larger than that of pores at the soil surface, while straining traps them in the necks of pores that have larger dimensions than the colloid itself (McGechan & Lewis 2002). Previous studies have shown that soil disturbance increases filtration and reduces pathogens in runoff (Dosskey *et al.* 2007), while straining has a significant effect on the transport and deposition of *E. coli* under saturated soil conditions (Bradford *et al.* 2006*a*). In accordance with those studies, high rates of *Giardia* reduction were observed in the current study, which involved both soil disturbance and saturated conditions. For example, high rates of *Giardia* reduction were observed after only a single growing season on the regenerating 'exotic grasses/weeds' treatment, while improvements of 79% were observed on the native treatments over the same time. This was considerably faster than previous estimates of up to three years to see such effects (Dosskey *et al.* 2007). Nevertheless, in the absence of soil porosity and grain size measurements, determining the particular mechanism responsible for the retention of *Giardia* in the four treatments remained unclear.

In order to detect mechanical filtration or pore exclusion, one of the most reliable methods available compares the temporal pattern of standardised concentrations of the conservative tracer with the colloid (C/Co) using breakthrough curves (Ginn *et al.* 2002). For example, a significantly higher colloid C/Co compared to that of the tracer in the rising limb to the peak indicates the colloid moved faster than the mean pore-water velocity, bypassing the soil matrix, and travelling via either overland flow or through macropores (Ginn *et al.* 2002). No such differences were observed between *Giardia* and bromide in the rising limb in the current study, hence mechanical filtration was unlikely to have been the mechanism responsible for *Giardia* reduction. Rather, given the *Giardia* to bromide ratios were less than 0.35 in the spring and 0.07 in the autumn, and displayed distinct tailing in all four treatments, it suggested both *Giardia* and bromide had travelled through the soil matrix. Therefore, while *Giardia* that entered the soil matrix were unlikely to be released due to straining, bromide could diffuse in and out. As such, similarities between the *Giardia* to bromide ratio curves from the four treatments indicated that the underlying mechanism was infiltration resulting from soil disturbance through vegetation development and was largely unaffected by the specific vegetation type or percentage of cover.

An important component not accounted for in the current study was the influence of manure on the mechanisms controlling *Giardia* deposition in the soil matrix. Factors governing the release of *Giardia* from faecal material during runoff events are currently unknown for flow rates above 10 ml per minute (Schijven *et al.* 2004). Consequently, the movement of *Giardia* across the landscape was investigated in our experiment using an artificially applied spike, as opposed to faecal material, to eliminate any confounding effects resulting from our much higher runoff rate of 2 L/min. However, manure particles may occupy sites useful for biocolloid straining (Bradford *et al.* 2006b) and thus affect *Giardia* retention. A recent study reported that as accessible straining sites become filled with colloids, less colloid deposition occurs, because colloids not yet retained are diverted from the previously available sites and remain in the runoff (Bradford *et al.* 2006a). It is possible a similar trend occurred in the current study, with the bulk of the *Giardia* cysts removed by straining until the available sites became full and cysts remaining unretained subsequently recovered in the collected runoff. While it is reasonable to assume some influence from manure deposition on *Giardia* removal, its effects remain unclear.

It seems likely that the overall decrease in the concentration of *Giardia* cysts in autumn runoff compared to spring runoff may equate to a lowered public health risk from contact with contaminated waterways. It should be noted, however, that the remaining cysts may still cause human infection, given that ingestion of as few as one to ten viable cysts can lead to an active infection (Cacciò *et al.* 2005). Nevertheless, on the basis of these results, it can be concluded that waterway fencing enabling the development of vegetation in the absence of stock grazing will create riparian buffers that can reduce the overall concentration of *Giardia* in overland surface runoff after a single growing season by as much as 98%. Thus, in the absence of planting, costs incurred for reducing *Giardia* in surface runoff contaminating waterways can be limited to that of fencing. However, if the additional cost of planting riparian buffers along such fence lines is not prohibitive, significant reductions in the volume of runoff containing *Giardia* reaching waterways in New Zealand can be gained by planting *C. richardii*, owing to the development of deeper tap roots within a single growing season.

CONCLUSIONS

The significant increase in dairy farming in Otago and the recent identification of *Giardia* strains in dairy cattle capable of causing infections in humans (Winkworth *et al.* 2008a) has highlighted the need for region-wide strategies to reduce waterway contamination (ORC 2005). While the Otago Regional Council's fencing initiative was primarily intended to address direct sources of pollution, the current study has shown the value of fencing in reducing input to waterways from diffuse pathogen sources. This result has more general, worldwide relevance. For example, the primary contribution of this study is to show that the fencing and retirement of land from grazing can effectively reduce the concentration of *Giardia* in runoff, with reductions of up to 98% observed after a single growing season. Although such reductions are roughly comparable to purposely planted vegetation buffers after one growing season, we have shown the added benefit of planting deep-rooted taxa (such as *C. richardii*) at producing significantly greater reductions after a single growing season by also decreasing the volume of runoff reaching waterways, than areas simply retired from grazing. While it is often impractical to establish planted buffers along all vulnerable waterways, various methods are available for site prioritisation (Tomer *et al.* 2003), ensuring maximum overall benefits are realised including increased biodiversity, bank stability, wildlife habitats and waterway shading.

ACKNOWLEDGEMENTS

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